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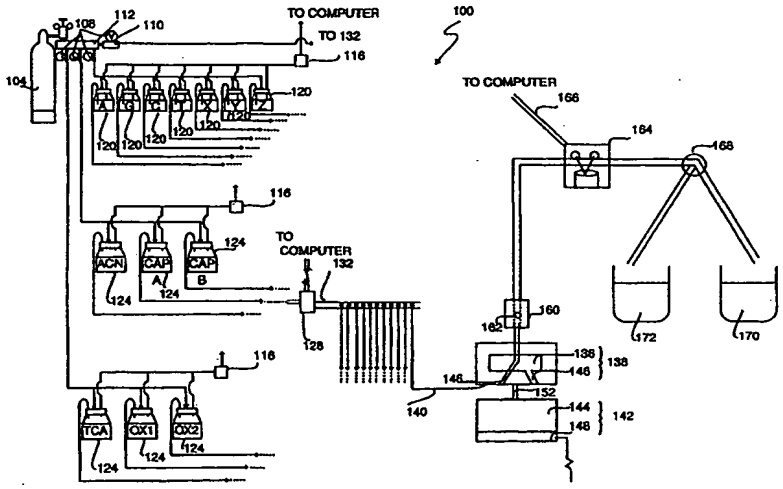

(57) Abstract

A system (100), method and computer program product for automated delivery of a fluid or gas. The invention includes a motor drive (142) system connected to a rotary valve (138) having a plurality of inlet ports and outlet ports (146). A plurality of vessels (120) containing fluids and/or gases are fluidly interconnected to the inlet ports of the rotary valve. Distribution valves (128) are provided which control the flow of fluids and/or gases form each of the vessels (120) to the inlet ports (146) on the rotary valve (138). A processor controls the distribution valves (128) to allow or restrict the flow of fluids and/or gases to the inlet ports and controls movement and positioning of the motor (142), thereby controlling rotation of the rotary valve (138).

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System, Method and Computer Program Product For Automated Fluid or Gas Delivery

Background of the Invention

Field of the Invention

The present invention relates to fluid or gas delivery systems, and more particularly, to multiple port fluid or gas delivery systems for use in an automated chemistry processing apparatus. More specifically, the present invention relates to an apparatus and method for performing automated chemical synthesis of oligonucleotides or peptides, and in particular, the synthesis of multiple different oligonucleotides or peptides in a concurrent manner.

Related Art

Many different types of processes require delivery of fluids and/or gases at various intervals and in various amounts. For example, in many chemical reactions, such as chemical synthesis, reactants are delivered to a reaction chamber in predetermined amounts and allowed to react before being flushed from the chamber. Examples of such chemical processes include: oligonucleotide synthesis (including derivative nucleotides and/or labeled nucleotides), peptide synthesis (including derivative amino acids and/or labeled amino acids), protein synthesis, synthesis of ordered-sequence biopolymers (e.g., conventional DNA, antisense DNA, RNAs, peptides, aptamers, diversomers, and polysaccharides), and DNA or biomolecule purification.

With respect to oligonucleotide synthesis, the goals of the Human Genome project has resulted in a number of current and emerging technologies requiring dramatically increasing numbers of oligonucleotides. Such technologies include: forward and reverse primers for the polymerase chain reaction (PCR), primer walking for DNA Sequencing, linkers for molecular

cloning, short oligonucleotides for DNA-DNA hybridization, polymorphism mapping, sequence tagged site (STS) mapping, radiation hybrid mapping, mutation detection using allele specific organization (ASO) and more recently, arrays of oligonucleotides on planar surfaces (CHIP Arrays), in which a single array may contain hundreds to thousands of oligonucleotides.

Existing commercial instruments for producing oligonucleotides are severely limited by five main factors: (1) scale of synthesis, (2) number of oligonucleotides which can be produced at one time, (3) quality of a standard synthesis, (4) reagent consumption, and (4) absence of communication software compatible with high throughput processing.

Typically, any of the aforementioned biological techniques use minute quantities (less than 5-50 picomoles) of oligonucleotide in each assay, yet existing commercial instruments produce 1,000-fold to 10,000-fold greater quantities than is needed. This translates to higher cost to produce an oligonucleotide since the reagents used in the synthesis are expensive. This cost is passed on to the end user performing the assays, and can result in prohibitively high costs which force the researcher to seek alternative technologies which may cause significant delays in time and results.

A variety of solid phase oligonucleotide synthesis techniques are known to those skilled in the art. Such techniques include phosphoramidite, phosphotriester, phosphodiester, phosphite and H-phosphonate methods and the like, each of which is generally known in the field of molecular biology. For example, the β–cyanoethyl phosphoramidite method is described in U.S. Patent 4,458,066 issued to Caruthers, *et al.*, entitled "Process for Preparing Polynucleotides," which is incorporated herein by reference.

An oligonucleotide is a macromolecule comprising a sequence of two or more nucleotides, each of which includes a sugar and a base. Each nucleotide is separated from adjacent nucleotides with an internucleotide linkage, which effectively serves to bond the nucleotides together. One process for oligonucleotide synthesis can be divided into four main segments, all of which are required to add a single nucleotide to one that is affixed to a solid support.

An oligonucleotide can be constructed by repeating these four segments multiple times to obtain the required length. Similarly, additional or other chemicals or reagents can be added to the process to produce derivative oligonucleotides which comprise derivative nucleotides and/or labeled nucleotides.

These four segments are: detritylation, coupling, capping, and oxidation. Typically, a 3′ nucleotide is attached to a solid support. During detritylation, a deprotectant is added to remove a protecting group known as a dimethoxytrityl (DMT) from the 5′ end of this attached nucleotide to thus "deprotect" or reveal a 5′ hydroxyl group. As a result, the last nucleotide in the sequence has one hydroxyl that is ready to receive a next phosphoramidite.

In the coupling segment, an excess of desired nucleotide (the second nucleotide specified in the oligonucleotide sequence), reacted with a mild acid to allow the activation of its 3′ phosphate, is added. The 3' phosphate in the second nucleotide bonds with the oxygen in the hydroxyl of the last nucleotide in the sequence, thus providing support-bound nucleotides. After the support-bound nucleotides are formed, excess nucleotides are flushed from the vessel with, for example, a wash solution such as acetonitrile (ACN). This results in the attachment of a second nucleotide to the first nucleotide.

The capping segment allows reaction of uncoupled nucleotides to be deactivated at their 5′ termini, thus preventing their participation in future coupling reactions. In particular, a capping agent is added to block all the unprotected hydroxyls from reacting with phosphoramidites introduced at a later stage. A wash solution, such as ACN, is again introduced to flush out the capping agent.

The oxidation segment is then applied to stabilize the internucleotide bond between the coupled bases. In one example, an oxidizing agent is added to convert a trivalent phosphorous to pentavalent. The oxidizing agent is then flushed with a wash solution.

All four of these segments are linked together to create a cycle, in which one cycle of synthesis results in the addition of one nucleotide to the nucleotide bound to the solid support. At the completion of the synthesis, the synthesized

oligonucleotide is removed from the solid support by treatment with a strong base, such as ammonium hydroxide.

A number of different bases can be used to form the oligonucleotide. The four most common bases are adenine, cytosine, guanine, and thymine (*i.e.*, A, C, G, and T, respectively). However, derivative or labeled nucleotides can be used in the process to make a variety of oligonucleotides. The internucleotide linkage is most commonly a phosphate, which may be substituted with a variety of substituents at a nonbridging oxygen atom, most commonly by sulphur or an alkyl, ester, or amide group.

Peptide and protein synthesis includes many of the same steps as oligonucleotide synthesis. In peptide synthesis, the deprotected amino terminus of a support-bound amino acid is reacted with the activated carboxyl terminus of an incoming amino acid, resulting in the formation of a peptide bond. The process of deprotecting the amino terminus, activating the carboxyl terminus, and performing the coupling reaction with the next amino acid is repeated until the desired amino acid sequence is completed.

A peptide is a molecule composed of at least two amino acids or derivative amino acids, such amino acids and amino acid derivatives include: alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutaminic acid, lysine, arginine, histidine, and derivatives thereof. Such amino acids and derivatives may be found in Lehninger, Biochemistry, Worth Publishers, New York, NY and in Stryer, Biochemistry, 3rd ed., WH Freeman and Co., New York, NY.

The peptide synthesis process is similar to the above-described oligonucleotide synthesis process, in that in solid phase synthesis, the peptide chains are assembled one amino acid at a time, with the first amino acid attached to a support, and the remaining amino acids added one-by-one, until the peptide chain is complete. During the process of adding additional amino acids many of the same segments as discussed above must be performed: deblocking, coupling (combined with activation), and capping.

In deblocking, a temporary α amino protecting group is removed using a reactant. The next protected amino acid is then coupled to the existing, deblocked amino acid. Once coupling is complete, the excess amino acid solution and reaction by-products are removed by washing. Once the peptide is assembled, it can be cleaved from the support and any side chain protected groups can be removed. The peptide may then be used without further isolation or may be purified prior to use.

These methods as well as others for producing oligonucleotides, peptides, proteins and the like are time consuming and the materials that are used are expensive and require special handling and disposal after being used.

It would be desirable to increase the number of oligonucleotides, peptides, and proteins that can be produced at one time, and to do so efficiently. Large-scale synthesis, however, raises several concerns. On the one hand, a high degree of homogeneity is desirable. On the other hand, competing concerns affect the efficient use of materials. For example, in oligonucleotide synthesis, efficient use of phosphoramidites and ACN is particularly desirable. While an excess amount of phosphoramidites is needed to ensure that as many as possible of the nascent oligonucleotides react with newly introduced phosphoramidites, the quantity of phosphoramidites introduced into the vessel should not be too excessive and wasteful. It is also desirable to reduce the amount of ACN that is used, while still flushing out, or at least diluting, leftover phosphoramidites as much as possible. If the flushing is insufficient, leftover phosphoramidites in the vessel or in various conduits leading to the reaction chamber can produce nonhomogeneous sequences.

Furthermore, as many of the synthesis steps are repetitive, efficiency can be increased by automation. For example, in an automated nucleic acid synthesis instrument, various steps are carried out by a reagent delivery system which dispenses a number of chemical reagents in a predetermined sequence in a cycle into a synthesis reaction chamber. Typically, the present generation of automated DNA sequential synthesizers place a derivatized solid support such as controlled pore glass (CPG), into an individual reaction chamber to provide a

stable anchor on which to initiate solid phase synthesis. Using a series of complex valving and pumps coupled to the reaction chamber, the appropriate selected reagents are sequentially filtrated through the chamber in a predetermined manner. Contact of the reagent with the polymer units pre-affixed to the CPG, which is retained and supported in the chamber by a sample support porous frit, causes a reaction resulting in sequenced growth thereon. In any case, for the purposes herein it is important to note that the reagents are delivered to the reaction chamber via several valves.

While each reaction chamber of such an assembly is effective to rapidly mass-produce a population of sequence defined oligonucleotides, the current assemblies are limited. The reaction chambers are placed within the automated apparatus so that chemicals can be added to the reaction chambers in sequence and in appropriate amounts in an automated fashion. Currently known automated synthesizers can produce only a few oligonucleotides at a time and are limited by the number of reaction chambers located within the machines. The number of reaction chambers is limited as a practical matter by the increased complexity of the plumbing and valving network as the number of reaction chambers increase since currently known synthesizers provide a tightly plumbed network from the several reagent supply reservoirs to each reaction chamber. Increased reaction chamber capacity is limited due to physical limitations of the valving configuration. Furthermore, a conventional synthesis apparatus is not generally amenable to integration with automation or other robotic lab instrumentation, and an operator must intervene to load and remove each individual reaction chamber manually which increases the opportunity for human error.

A more important limitation of the synthesis process is that all reagents are funneled through a common manifold passage. Only one reagent or combination thereof, thus, can be simultaneously deposited in select reaction chambers. In addition, for each independent synthesis or reaction, the common manifold passage and associated valving must be flushed with a cleaning reagent so that residual phosphoramidite or deblocking reagents will not be undesirably

deposited in a reaction chamber. This approach wastes time and increases operating costs.

Moreover, the synthesis efficiency depends in large part on the integrity of the reagents. In fact. the effectiveness of the synthesis process is very sensitive to the purity of the reagents. Cross contamination between different reagents often adversely affects the chemical integrity of the reagents and thus the efficiency of the controlled chemical reactions that involve the reagents. One source of cross contamination is in the valves, particularly multi-port valves which select delivery between different reagents. There are inevitable dead volumes in the valves associated with switching between reagents. Therefore, a design concern for fluid delivery systems designed for handling several types of fluids in a system, is the reduction of cross contamination between fluids. For this reason, past instrument designs which have required absolute control over cross-port contamination and random selection of chemical reagents have avoided use of multi-port valves because of the difficulties in preventing such contamination.

DNA or biomolecule purification involves flushing reagents through a reaction chamber simultaneously for purification. Although in this application, cross-contamination of reagents is not as much of a concern, it would be preferable to be able to efficiency perform large-scale and/or multi-sample purification. Conventional purification systems use robots to empty pipettes of reagents into vessels containing the material to be purified. The reagents are then evacuated from the vessels using a vacuum. Although purification using these systems can largely be performed without much human intervention, the total throughput of these conventional systems is limited because the robots must add each reagent separately to a multitude of vessels. It would be preferable to have a fluid and/or gas delivery system that could quickly and efficiency deliver fluids and/or gases to the reaction chamber in an automated fashion to increase total throughput.

Summary of the Invention

The present invention is designed to deliver fluids and gases to a vessel, tube, well or chamber, such as a reaction chamber housing a solid support, for a variety of purposes. For example, the present invention can be used to deliver fluids and/or gases to a reaction chamber for: oligonucleotide synthesis (including derivative nucleotides and/or labeled nucleotides), protein synthesis (including derivative amino acids and/or labeled amino acids), synthesis of ordered-sequence biopolymers (e.g., conventional DNA, antisense DNA, RNAs, peptides, aptamers, diversomers, and polysaccharides), or for DNA or biomolecule purification. Further, as would be apparent to one skilled in the relevant art, the present invention can be used to deliver fluids and/or gases in a variety of other applications.

With respect to oligonucleotide synthesis, the present invention can be used to allow oligonucleotides to be synthesized in the picomole to micromole range. Further, the system of the present invention provides for parallel processing to enable simultaneous synthesis of between 1-200 oligonucleotides at a time and produce high quality oligonucleotides which require no post-synthesis purification, allowing direct usage in many biological assays. The apparatus of the present invention can be adapted or reformatted to increase the number of different oligonucleotides made at a time.

An advantage of the present invention is that it results in a lower cost per oligonucleotide or peptide due to reduced scale and reagent consumption and reduces the time needed to produce such oligonucleotides or peptides. Further, the present invention allows active, real-time quality control of the synthesis process by an integrated output monitoring system.

An overall software control system allows incoming customer orders to be automatically transferred to a host computer that controls the entire mechanical process, and amasses and analyses output data for display on a graphical user interface.

The advantages noted for oligonucleotide synthesis also would be recognized when performing other applications (e.g., peptide synthesis, polysaccharide synthesis, DNA or RNA purification, and like applications) with the present invention. These advantages include performing multiple simultaneous reactions, lower cost due to less waste of reagents, real-time quality control, reduced contamination, higher throughput due to shorter cycle times, and automated control.

The present invention provides a system for fluid or gas delivery that uses considerably less reagent material for equivalent yields and eliminates the need for flushing the fluid path. The passageways of the present invention are extremely short and smooth with little or no measurable dead volume and/or backflow and are designed to pre-mix chemicals when required. The present invention can deliver exact amounts of reagents (to a fraction of a microliter) to the reaction chamber or solid support in the correct order without diverting any to waste, thereby saving time and reagents. Further, the automation of the process reduces labor costs in the operation, provides excellent process control and quality control and enables the production of significant numbers of products or reactions each day.

The system of present invention includes vessels for storing starting reagents, chemicals, or other fluids for the reaction. These vessels are preferably pressurized with an inert gas. Each vessel is fluidly connected to its own distribution valve. Implementation of the present invention is foreseen to include simultaneous synthesis of 1-200 or more oligonucleotides, peptides, and the like. Thus, the vessels may be used to supply more than one, and preferably many, reaction chambers. As such, the vessels are preferably connected via their distribution valves to a manifold such that the chemicals can be supplied to more than one, and preferably many, reaction chambers at a time.

A rotary valve assembly is preferably attached via tubing to the manifold, although the rotary valve may be connected directly to vessels containing the starting reagents, chemicals, etc. The positioning of a valve rotor within the rotary valve assembly is controlled by a computer-controlled motor drive system.

The reaction chamber is preferably connected to the rotary valve assembly. An output monitor is disposed about the tubing after the reaction chamber to monitor the effluent as it leaves the reaction chamber. However, monitors could be placed at any point or multiple points in the system of the present invention to monitor the process. The data from the monitor is fed back into the host computer.

The rotary valve assembly includes a stationary hollow body member having multiple inlet ports in the base of the body member and a cylindrical valve rotor disposed within the body member. The valve rotor has at least one communicating port which may be selectively positioned in alignment with a selected inlet port in the base of the body member. A passageway in the valve rotor connects the communicating port with the reaction chamber. The inner surface of the body member and the outer surface of the valve rotor are provided with uninterrupted smooth cylindrical surfaces.

Additional features of the rotary valve assembly include O-rings, which may be disposed around each inlet port and retained in the desired position by a recess in the base of the stationary housing. O-rings could alternately be disposed in the valve rotor. A slight pressure is exerted on the rotary body member thereby uniformly compressing the O-rings in an axial direction at all points about their peripheries thus providing a sealed interstitial space. The O-rings aide in providing a functionally leak proof valve. Other ways to provide a functionally leakproof valve include using a valve rotor made of a TEFLON® material, ceramic or glass. Such a design if preferably used without O-rings.

Further features preferably include a check valve integrated into the passageway in the valve rotor such that the ball of the check valve alternates between a closed and open position. The check valve is positioned in the passageway so that it is adjacent each inlet port to prevent backflow. Alternatively, the check valve may be located within the inlet port. The passageway above the check valve is filled and/or flushed with a fluid or a gas as the valve rotor is rotated through the valve positions. The check valve reduces the contamination ratio by minimizing backflow of fluids into the inlet ports.

Another feature of the valve rotor of the present invention is that the volume and shape of the passageway is designed to improve mixing of fluids prior to entering the reaction chamber. Mixing is further achieved in a swirl chamber which is provided at the end of the passageway and before the reaction chamber, although the swirl chamber could be positioned at any point in the passageway. The volume of the swirl chamber can be adjusted by design so that for certain chemistries complete mixing of the chemicals takes place prior to entering the reaction chamber.

For example, in oligonucleotide synthesis, at one step of a typical synthesis cycle, a phosphoramidite is selected and a small amount is pulsed into the passageway in the valve rotor. The valve rotor then moves to the activator position and a small amount of activator is pulsed into the passageway. The system continues alternating pulses of phosphoramidite and activator such that as the pulses are pushed up or through the passageway toward the reaction chamber the two fluids are mixed. When the fluids reach the swirl chamber, they are further mixed, as the conical configuration of the swirl chamber causes the fluids to swirl about the chamber before entering the reaction chamber. When mixing of chemicals is not desired, slug flow (i.e., the flow of a liquid such that little mixing occurs between several fluids moving through a passageway) can easily be achieved by repetitively delivering longer pulses of only a single fluid through the passageway.

The motor drive system may include a stepper motor and an electronics package. The electronics package controls drive circuits and may also monitor one or more position detectors. Alternately, the motor drive system may include servo motors with encoders to determine the angular position of the motor and/or resolvers to determine the exact position of the motor.

The output monitor of the present invention is disposed in the system after the reaction chamber to monitor the effluent from the reaction chamber. Similar monitors may also be disposed at other locations in the system. The output monitor preferably includes an optical sensor and a detector assembly located on opposite sides of a portion of the tubing which leads from the reaction

chamber to a waste disposal. The optical sensor preferably includes two LEDs of differing wavelengths. The output monitor further includes a LED driver to control illumination of the LEDs. Narrow slits disposed between the optical sensor and the tubing on one side and between the detector assembly and the tubing on the other side, allow the light from the LEDs to pass from the LEDs through the tubing and to the detector. A signal processor processes the signals received by the detector assembly. A feedback loop sends the signals back to the host computer so that continuous monitoring of the effluent can be used to implement instantaneous corrective measures during the synthesis process, when needed. The monitor may also serve to analyze the type and quality of the product produced.

A level sensor on the storage vessels may be provided to indicate to the host computer when one of the storage vessels is empty or near empty. A LED is preferably placed on one portion of sidewall such that the LED is located within a curved portion of the sidewall. A detector is positioned opposite the LED on the opposite curved portion of sidewall.

When there is liquid in the storage vessel, the light from the LED travels directly across the storage vessel to the detector. However, when there is no liquid above the light from the LED, the light is refracted because the index of refraction of the liquid is different from the index of refraction of air (e.g., no liquid). In this case less of the light from the LED reaches the detector. As such, an output signal is sent from the detector to the host computer that one of the storage vessels is empty or near empty. The host computer can either signal an alarm to the user to refill the particular storage vessel which is empty, or it can send a signal to the system to automatically refill the storage vessel from a stock bottle.

The computer program controls the process of the present invention using the following steps: receiving an input of a desired reaction (e.g., a desired nucleotide sequence for oligonucleotide synthesis, a desired amino acid sequence for peptide synthesis, or a desired list of reagents for purification), creating an event matrix of the events necessary to produce the desired sequence or reaction,

rotating the valve rotor to a destination based on the event in the event matrix, energizing a distribution valve for a particular reagent for the event to deliver the reagent to the reaction chamber, executing a time delay for the distribution valve, de-energizing the distribution valve at the end of the time delay, and repeating these steps for each event in the matrix until the desired sequence is synthesized or the desired reaction is accomplished.

The present invention provides for faster processing because the contamination levels are so low that little or no flushing is required between valve rotor positions. Further, the present invention allows for the chemistry or reactions to be controlled with a simple sequential control string from a computer. This saves time and uses less reagents as the amount of chemical or reagents delivered to the rotary valve can be optimized such that only the desired minimum amount is used. Such a valve is ideally suited for oligonucleotide synthesis and peptide synthesis as minimum contamination yields high efficiencies.

Although the present application mainly focuses on use of the present invention for a particular application, i.e., oligonucleotide synthesis, it will be apparent to one skilled in the relevant art that the fluid and gas delivery system of the present invention can be used for a variety of other processes. For example, the system of the present invention could be used as a DNA or RNA purification system, a biomolecule purification system (such as for proteins, hormones, co-factors, and the like), or for biopolymer synthesis. Further, the system of the present invention could be used to make nucleotide derivatives or labeled nucleotides or amino acid derivatives or labeled amino acids.

Further features and advantages of the invention as well as the structure and operation of various embodiments of the present invention are described in detail below with reference to the accompanying drawings. Other embodiments of the present invention will be apparent to one of ordinary skill in the art in view of the following drawings and description of the invention.

Brief Description of the Figures

The features and advantages of the present invention will become more apparent from the detailed description set forth below when taken in conjunction with the drawings in which like reference numbers indicate identical or functionally similar elements. Additionally, the left-most digit of a reference number identifies the drawing in which the reference number first appears.

FIG. 1 is a schematic diagram showing an embodiment of the delivery system of the present invention.

FIG. 2 is a side view of a first embodiment of a rotary valve assembly, a stepper motor and a reaction chamber of the present invention.

FIG. 3 is a sectional view of the rotary valve assembly and the reaction chamber, taken along a line 3-3 in FIG. 2.

FIG. 4 is a sectional view of the rotary valve assembly taken along a line 4-4 in FIG. 2.

FIG. 5 is a partial sectional view of a motor drive system of the present invention.

FIG. 6 is a block diagram of an output monitor of the present invention.

FIG. 7 is a diagram of a portion of the output monitor of FIG. 6.

FIGS. 8A-8C are sequence tables used in an exemplary synthesis process of the present invention.

FIG. 9 is an event matrix used in the exemplary synthesis process of the present invention.

FIGS. 10A, 10B and 10C are high level flow charts of a method for performing a reaction in accordance with the present invention.

FIG. 11 is a Graphical User Interface (GUI) for use in loading the sequences in the present invention.

FIG. 12 is a GUI for use in monitoring the output of the system of the present invention.

FIG. 13 is a GUI for use in saving the data from the output monitor in the present invention.

FIG. 14 is an example of a computer system used to implement the present invention.

FIGS. 15A and 15B show an exemplary graph of an HPLC analysis of a 17 base-long oligonucleotide and a table of the results, respectively, synthesized according to the present invention.

FIGS. 16A and 16B show an exemplary graph of an HPLC analysis of a 30 base-long oligonucleotide and a table of the results, respectively, synthesized according to the present invention.

FIG. 17 shows a level sensor system of the present invention.

FIG. 18 is a sectional view of a second embodiment of the rotary valve assembly taken along a line 4-4 in FIG. 2.

FIG. 19 shows the continuous output from output monitor during a synthesis process using the present invention.

FIG. 20 shows an exploded view of a portion of the rotary valve assembly of the present invention.

FIG. 21 shows a perspective view of an alternate embodiment of the system of the present invention.

FIG. 22 shows a partial sectional side view of the system of the alternate embodiment shown in FIG. 21.

FIG. 23 shows a partial sectional top view of the system of the alternate embodiment shown in FIG. 21.

Detailed Description of the Preferred Embodiments

I. Overall System

FIG. 1 shows the overall configuration of a system 100 of an embodiment of the present invention. Referring to FIG. 1, a compressed gas cylinder 104 provides an inert gas under pressure through various pathways in system 100. In one embodiment, the inert gas is helium. In an alternate embodiment, the inert gas can be argon or nitrogen.

The pathways in system 100 are pressurized to facilitate the flow of liquids through system 100. The inert gas is also used to keep the chemicals dry. In one embodiment, the pressure of system 100 is maintained at about 7.5 psi in all pathways. In some cases, however, it may be advantageous to increase or decrease the pressure on some or all of the pathways depending on the density and/or amount of the fluid to be supplied.

In one embodiment, the pressure in a pathway carrying a wash solution such as ACN could be higher than 7.5 psi because larger quantities of wash solution are used in the synthesis process than are used of the other reagents. By increasing the pressure in this pathway, the amount of time it takes to deliver the wash solution to the reaction chamber is decreased. On the other hand, the pressure in a pathway carrying phosphoramidites or activator (TET) could be lower than 7.5 psi, preferably 1-4 psi, to reduce and better control the amount of reagent delivered during a given time interval.

In one embodiment, computer-controlled regulators 108 may be used to monitor and maintain the pressure in the pathways leading to the various fluids needed for synthesis. As shown in FIG. 1, system 100 includes three regulators 108. However, it would be apparent to one skilled in the relevant art that more or less regulators can be used as long as the gas flow rate is controllable.

In one embodiment, computer-controlled regulator 110 may also be used to monitor and maintain the pressure in a manifold 132 which distributes the fluids and gases to various rotary valve assemblies, as discussed in further detail below.

A gas distribution manifold 112 is used to distribute the inert gas from gas cylinder 104 to computer-controlled regulators 108 and computer-controlled regulator 110. A computer-controlled vent valve 116 is used for back flushing chemicals into their storage vessels 120 and 124 for times when the system is not in use or for filling working vessels from stock vessels. (The lines for remote filling of vessels are not shown in FIG. 1.) Although FIG. 1 shows three vent valves 116, it would be apparent to one skilled in the relevant art that fewer or more vent valves can be used depending on the configuration of the system. For example, each vessel could be connected to its own vent valve 116. Alternately, a single vent valve 116 could be used for all of the storage vessels.

System 100 uses vessels 120 for the phosphoramidites, although other chemicals or reagents may be used depending on the need. As shown in the embodiment of FIG. 1, the first four vessels 120 contain adenine (A), guanine (G), cytosine (C), and thymine (T). The remaining three vessels 120 are reserved for other phosphoramidites, or other reagents which are referred to in FIG. 1 by a generic nomenclature X, Y and Z.

System 100 uses vessels 124 for the additional chemicals which are used during synthesis process. In the embodiment shown in FIG. 1, vessels 124 contain reagents, such as CAP A and CAP B, which are capping reagents used in the capping segment to allow reaction of uncoupled nucleotides to be deactivated at their 5' termini. Vessels 124 further contain reagents, such as OXID 1 and OXID 2, which are used during an oxidation segment, to stabilize the internucleotide bond between coupled bases, and TCA, which is used during the detritylation segment to remove a protecting group from the 5' end of the nucleotide. The chemical ACN, also contained in vessels 124, is a wash solution that may be used between applications of other reagents to flush the system. These particular chemicals are shown by way of example only. It would be apparent to one skilled in the art of oligonucleotide or peptide synthesis that different chemicals and reagents could be used depending on the particular application and synthesis process being employed.

For example, other chemicals could be added to produce derivative nucleotides or labeled nucleotides and/or oligonucleotides containing such derivative and/or labeled nucleotides. As used herein "nucleotide" refers to a base-sugar-phosphate combination. Nucleotides are monomeric units of a nucleic acid sequence (DNA or RNA). The term nucleotide includes deoxyribonucleotide triphosphates, such as dATP, dCTP, dITP, dGTP, dTTP, or derivatives thereof. The term nucleotide as used herein also refers to dideoxyribonucleotide triphosphates (ddNTPs) and their derivatives and ribonucleotide tripphosphates, such as ATP, UTP, GTP, CTP, or derivatives thereof. Examples of ddNTPs include, but are not limited to, ddATP, ddCTP, ddGTP, ddITP and ddTTP.

Examples of nucleotides and derivatives thereof which can be made using the present invention include, but are not limited to, dUTP, dATP, dTTP, dCTP, dGTP, dITP, 7-deaza-dGTP, α-thio-dATP, α-thio-dTTP, α-thio-dGTP, α-thio-dCTP, ddUTP, ddATP, ddTTP, ddCTP, ddGTP, ddITP, 7-deaza-ddGTP, α-thio-ddATP, α-thio-ddTTP, α-thio-ddGTP, α-thio-ddCTP or derivatives thereof. The nucleotides may be unlabeled, or they may be detectably labeled by coupling them by methods known in the art with radioisotopes (e.g., ^{3}H, ^{14}C, ^{32}P or ^{35}S), vitamins (e.g., biotin), fluorescent moieties (e.g., fluorescein, rhodamine, Texas Red or phycoerythrin), chemiluminescent labels, dioxigenin, bioluminescent labels, enzyme labels and the like.

Each vessel 120 and 124 is connected to compressed gas source 104 via regulators 108 such that the chemicals are pressurized in the vessels. Each vessel 120 and 124 is also fluidly connected to its own distribution valve 128. For ease of illustration, only one distribution valve 128 is shown in FIG. 1. However, each dashed line extending from vessels 120 and 124 is intended to be connected to its own distribution valve 128. In one embodiment, distribution valve 128 is a solenoid valve. Distribution valve 128 is preferably made using a TEFLON® material, available from E. I. Du Pont de Nemours, that has been rated to be used with the types of reagents typically used in the synthesis process. However, it would be apparent to one skilled in the relevant art that distribution valve 128

could be made using other materials, depending on the particular reaction or process being performed.

Implementation of the present invention is foreseen to include simultaneous synthesis of several oligonucleotides. In one example, DNA and RNA synthesis (e.g., oligonucleotide synthesis) can be simultaneously performed in eight separate rotary valve assemblies. In another embodiment, the system of the present invention is configured to concurrently produce 1-200 oligonucleotides. In still another example, the system is configured to produce 96 oligonucleotides at the same time. In these cases, vessels 120 and 124 may be used to supply more than one rotary valve assembly 138, as described below.

As such, as shown in FIG. 1, each vessel 120 and 124 is fluidly interconnected via its distribution valve 128 to a manifold 132 such that the phosphoramidite or chemical in vessel 120 and 124 can be supplied to more than one rotary valve assembly at a time. In one embodiment, manifold 132 is made from stainless steel that has been coated with silica, such as Silcosteel®, available from Restek Corporation, Bellefonte, PA. The silica coating is used to make the steel inert when it is used with reagents typically used in the synthesis process. It would be apparent to one skilled in the relevant art that other materials could be used for manifold 132, depending on the fluids and/or gases being passed through the manifold for the particular reaction being performed.

In the embodiment shown, distribution valve 128 is computer-controlled and allows each of the pressurized chemicals to flow into its own manifold 132 for distribution to the appropriate rotary valve assembly 138 via tubing 140. For ease of illustration, manifold 132 is shown connected to only one inlet port 146 of rotary valve assembly 138. However, output from manifold 132, shown in FIG. 1 by dashed lines, is intended to supply either another inlet port 146 of rotary valve assembly 138 and/or inlet ports on additional rotary valve assemblies (not shown). In one embodiment, output from manifold 132 is fluidly connected to eight or more (preferably 12, 24, 48, 96, 192, 384, etc.) different rotary valve assemblies 138.

In one embodiment, tubing 140 is made of TEFLON® tubing having a 0.031 inch inner diameter and a 0.062 inch outer diameter, although other types and sizes of tubing may be used. Tubing 140 is preferably clear so that measurements of the effluent can be made directly through the tubing. Manifold 132 distributes the chemicals evenly to each rotary valve assembly 138.

In an example of system 100, when the synthesis process calls for a wash solution, such as ACN, all distribution valves 128 are turned off. Then, for those rotary valve assemblies 138 that require ACN, the valve rotor 136 is rotated to the ACN position (as is shown and discussed in FIGS. 4 and 5 below). The distribution valve 128 connected to the ACN vessel 124 is then turned on, allowing the pressurized ACN to flow from vessel 124, through manifold 132. For those valve rotors 136 that are in the ACN position, ACN will flow through valve rotor 136 and through reaction chamber 160. For those valve rotors 136 that are in a different position, no ACN will flow through that valve rotor 136. In this way, each valve rotor 136 is independent of each other.

As explained in further detail below with reference to FIG. 4, each rotary valve assembly 138 is connected to a motor drive system 142. One embodiment of motor drive system 142 is shown in further detail in FIG. 5, and includes a stepper motor 144 and an electronics package 148. In one embodiment, stepper motor 144 is a 200 step Superior SLO-SYN® stepper motor available from Warner Electric Motors and Controls Division, Ann Arbor, MI as model #KML061S02E. Electronics package 148 controls drive circuits (not shown in FIG. 1) and a zero position detector (not shown in FIG. 1). A shaft 152 extends from stepper motor 144. Shaft 152 may have an extension added to one end so that a square drive shaft (not shown) can be added that allows indexing to the motor fields, insuring accurate and reproducible alignment of valve rotor 136 with inlet ports 146 of a rotary valve assembly 138. Motor drive system 142 will be described in further detail below, with respect to FIG. 5.

In another embodiment, motor drive system 142 can include servo motors with encoders. Although such a motor drive system would be more complex and costly, it would be apparent to one skilled in the relevant art how to construct

such a motor drive system to control the position of valve rotor 136 of rotary valve assembly 138.

Rotary valve assembly 138 includes a valve rotor 136 and inlet ports 146. Valve rotor 136 is fixedly attached to one end of shaft 152 so that it can be rotated to align with inlet ports 146. Rotary valve assembly 138 is connected to a reaction chamber 160. In one embodiment, control pore glass (CPG) 162 is disposed in reaction chamber 160. Such a reaction chamber is the Perceptive column, available through ABI/Perseptive of Foster City, CA. Another such reaction chamber is the DNA Synthesis Column available through Solid Phase Sciences of Novato, CA. It would be apparent to one skilled in the relevant art, that the support can be a solid or semi-solid support made of any material or combination of materials including, plastic, glass, agarose, metal, nitrocellulose, acrylamide, silica, nylon, cellulose, diazocellulose, modified polystyrene, polyvinyl chloride, polyethylene, dextran, polyvinyl fluoride, sepharose, polyacrylamide, latex, starch, polyvinyl toluene, polysaccharide, acrylic polymers, hydroxyapetite, and the like. In particular, the support matrix is made from a resin, such as polystyrene, polyacrylamide. acrylamide-impregnated silica or porous glass. The form of such supports may vary from beads, particles, filters, columns and the like. In another aspect. the reaction chamber can be replaced with any tube, vessel. or other container for collecting the fluid or gas supplied through the system 100.

The output from reaction chamber 160 is connected to an output monitor 164 for measurement of the trityl and reagents in the effluent. Measurements made by the output monitor are output via a line 166 to a host computer (not shown in FIG. 1).

A waste diverter valve 168 controls separation of the waste into a halogenated waste receptacle 170 and a non-halogenated waste receptacle 172. Separation of the waste is desirable as disposal costs for halogenated and non-halogenated waste differ significantly.

II. Rotary Valve Assembly

FIG. 2 is a side view of rotary valve assembly 138, motor drive system 142 and reaction chamber 160 of an embodiment of the present invention. Referring to FIG. 2, tubing 140 is shown connected to inlet ports 146 of rotary valve assembly 138. Only two inlet ports 146 are shown being connected to tubing 140 in FIG. 2. However, in one embodiment of the present invention each of inlet ports 146 is connected via tubing 140 to a corresponding reagent manifold 132.

A. Structural Assembly

Referring to FIG. 3, a partial cross-section of rotary valve assembly 138, taken along a line 3-3 in FIG. 2, is shown. Reaction chamber 160 is disposed on top of rotary valve assembly 138 and includes a support column (not shown) and a solid support (not shown). In one embodiment, the solid support is CPG. However, as discussed above, it would apparent to one skilled in the relevant art that a variety of reaction chambers or supports could be used depending on the particular application.

Rotary valve assembly 138 is formed of a first portion 306 and a second portion 308. First portion 306 is a hollow stationary body having a smooth uninterrupted inner surface 310 that is cylindrical in shape. In one embodiment, first portion 306 is made from stainless steel or aluminum. The material for this part is not coated because it does not come in direct contact with the reagents in this embodiment. In one embodiment, second portion 308 is made from stainless steel that has been coated with silica, such as Silcosteel®, available from Restek Corporation, Bellefonte, PA. The silica coating is used to make the steel inert when it is used with reagents typically used in the synthesis process. It would be apparent to one skilled in the relevant art that other materials could be used for second portion 308, depending on the fluids and/or gases being passed through the manifold for the particular reaction being performed.

Second portion 308 is secured by locating pins 312 to first portion 306, and forms part of the stationary construction. Second portion 308 has a smooth upper surface 314 and an aperture 316. The sidewalls 318 of second portion 308

about aperture 316 include a shaft bearing 358. Shaft 152 from stepper motor 144 extends through aperture 316 and is slip fit into a recess 320 in a valve rotor 136 to engage valve rotor 136. Bearing 358 is provided to facilitate rotation of shaft 152 within aperture 316.

Second portion 308 further has inlet ports 146. Although only two inlet ports 146 are visible in the cross-section of FIG. 3. in one embodiment, second portion 308 has twenty inlet ports 146, where one of the twenty inlet ports 146 representing the "OFF" position of the valve is not fluidly connected to a corresponding port of valve rotor 136. It would be apparent to one skilled in the relevant art that any number of inlet ports could be formed on the rotary valve to accommodate a variety of fluids or gases.

As shown in FIG. 3, one of the inlet ports 146 is substantially vertical and the other inlet port 146 is disposed in second portion 308 at an angle to the vertical. In one embodiment, inlet ports 146 alternate in this way, so that all of the inlet ports fit about the rotary valve assembly. In one embodiment, there are twenty inlet ports 146 about upper surface 314. The ends of inlet ports 146 terminate at upper surface 314 of second portion 308 such that they all lie in a circular path about a central axis of the rotary valve assembly.

A communicating port 326 is formed in a lower surface 328 of valve rotor 136. As valve rotor 136 is rotated by shaft 152 of stepper motor 144, communicating port 326 aligns with, and thereby allows fluid communication with, inlet ports 146. In one embodiment, valve rotor 136 is made from stainless steel that has been coated with silica, such as Silcosteel®, available from Restek Corporation, Bellefonte, PA. The silica coating is used to make the steel inert when it is used with reagents typically used in the synthesis process. It would be apparent to one skilled in the relevant art that other materials could be used for valve rotor 136, depending on the fluids and/or gases being passed through the manifold for the particular reaction being performed. For example, as described above, in an alternate embodiment, valve rotor 136 could be made of a TEFLON® material, or of ceramic or glass.

Upper surface 314 of second portion 308 is provided with recesses 330 about the ends of inlet ports 146. In one embodiment, O-rings 332 are seated in recesses 330 to provide a seal around inlet ports 146 to prevent contamination of communicating port 326 or lower surface 328 of valve rotor 136. Recesses 330 act to accurately locate O-rings 332 so that they are concentric with inlet ports 146 in second portion 308. Further, recesses 330 prevent displacement of O-rings 332 from their aligned positions during rotation of valve rotor 136. O-rings 332 assist in reducing contamination of the liquid flowing to the reaction chamber by sealing around inlet ports 146, such that when valve rotor 136 rotates from one inlet port 146 to another, the reagents from the inlet ports are not contaminated from reagents from other inlet ports. However, it would be apparent to one skilled in the art that the present invention could be implemented without using O-rings 332 and corresponding recesses 330 about inlet ports 146, as discussed in further detail below.

Second portion 308 also includes a recess 334 in upper surface 314 along a periphery of shaft 152 of stepper motor 144. Second portion 308 further includes a second recess 336 in upper surface 314 which forms a concentric circle about all of the inlet ports 146. O-rings 338 and 340 are seated in recesses 334 and 336, respectively, to provide a leak-proof valve. In one embodiment, O-rings 332, 338 and 340 are made of Kalrez® 3018 material, a perfluoroelastomer part, currently available from Dupont Dow Elastomers, L.L.C., Newark, Delaware.

Valve rotor 136 is disposed within first portion 306 and is provided with a smooth cylindrical outer surface 342. In one embodiment, valve rotor 136 is disposed within first portion 306 such that its smooth outer surface 342 does not come in direct contact with inner surface 310 of first portion 306. Lower surface 328 of valve rotor 136 slightly compresses O-rings 332, 338 and 340 into each of their respective recesses. As will be apparent, this results in compression of O-rings 332, 338 and 340 about their peripheries. In a preferred embodiment of the invention, the compression about the periphery of each O-ring 332, 338 and 340 is uniform.

Tubing 140 is connected to inlet ports 146 to selectively deliver fluids and/or gases to valve rotor 136 by rotating valve rotor 136 until it aligns with the appropriate inlet port 146. An alternate embodiment of system 100, in which distribution valves 128 are connected to inlet ports 146 via a distribution plate and manifold blocks is described in detail below with reference to FIGs. 21-23.

Communicating port 326 of valve rotor 136 is connected to a passageway 348. Passageway 348 extends through valve rotor 136 from communicating port 326 to reaction chamber 160. In one embodiment, the volume of passageway 348 is such that it holds at least double the volume of fluid in the smallest pulse of fluid delivered by the system through inlet port 146. For example, in one embodiment, the volume of passageway 348 is between 10-1000 μL. In another embodiment, the volume of passageway 348 is between 50-500 μL. In yet another embodiment having a smallest pulse of fluid of 38 μL, the volume of passageway 348 is between 76-100 μL.

In another embodiment, the volume of passageway 348 is such that it is at least half of the volume of the reaction chamber. For example, in one embodiment, the ratio of the volume of passageway 348 to the volume of reaction chamber 160 is within the range of 2:1 to 1:1. As such, in this example, if the volume of reaction chamber 160 is 100 ml, the volume of passageway 348 would be between 50-100 ml. This helps to insure proper mixing before the fluids reach reaction chamber 160.

A check valve 344 is disposed in passageway 348. The ball of check valve 344 alternates between contact with communicating port 326 and a plug 345, which is disposed above check valve 344 to limit the range of motion of the ball of check valve 344. In one embodiment, the ball of check valve 344 is made of a TEFLON® material. However, it would be apparent to one skilled in the relevant art that the ball of check valve 344 could be made of other materials, such that the ball can be made as close as possible into a substantially perfect sphere to provide a good seat against communicating port 326. Examples of other materials include: steel, stainless steel, sapphire, ceramic, ruby or synthetic ruby.

In the embodiment of FIG. 3, O-rings 332, 338 and 340 create a small interstitial 2004 space, as shown in FIG. 20, between valve rotor 136 and second portion 308 which may be filled and/or flushed with a wash fluid, such as ACN, as valve rotor 136 is rotated through different valve positions. Check valve 344 is provided to prevent backflow of fluid from passageway 348 to this interstitial space 2004 or to inlet ports 146.

An exploded view of the intersection between communicating port 326 and inlet port 146 is shown in FIG. 20. As shown in FIG. 20, the size of communicating port 326 below check valve 344 is minimized so as to reduce contamination. Further, an area 2008 between the sides of O-ring 332 is minimized to reduce the area in which any backflow from communicating port 326 may collect.

Returning to FIG. 3, passageway 348 connects communicating port 326 with an outlet port 346. Outlet port 346 is in fluid communication with reaction chamber 160. Fluid or gas flowing through communicating port 326 travels past check valve 344 and through passageway 348 into a swirl chamber 350. Swirl chamber 350 is conical in shape and is also designed to hold a volume of fluid at least double the volume of fluid in the smallest pulse of fluid delivered by the system through inlet port 146. In the embodiment shown in FIG. 3, swirl chamber 350 is at one end of passageway 348. However, swirl chamber 350 could be located at any point within passageway 348.

As the fluid travels through passageway 348 and through swirl chamber 350, the shape of swirl chamber 350 causes the fluids in the valve rotor 136 to swirl and thereby mix before exiting through outlet port 346. It would be apparent to one skilled in the relevant art that a commercially available static mixer could also be used, in place of or in addition to swirl chamber 350 to cause mixing of the fluids in valve rotor 136.

In another embodiment, check valve 344 may be disposed in each inlet port 146. In such an embodiment, the inlet ports would act like an injector, wherein a check valve would be spring-loaded and would open only when the communicating port of the valve rotor aligned with it. In this embodiment,

although no substantial backflow of fluid could enter inlet ports 146, backflow could occur from passageway 348 to the interstitial space 2004 between valve rotor 136 and second portion 308. It is preferable in this embodiment to use an inlet port configuration as shown in FIG. 18, as discussed in further detail below, and to avoid the use of O-rings in order to minimize any interstitial space between valve rotor 136 and second portion 308, thereby minimizing contamination.

First portion 306 is further provided with an aperture 352 on an upper end thereof. Outlet port 346 is disposed in aperture 352 and is in fluid communication with passageway 348 and reaction chamber 160. As shown in FIG. 3, valve rotor 136 has a recess 354 provided adjacent swirl chamber 350 and at the start of outlet port 346. An O-ring 356, similar to those described above, is seated in recess 354 to prevent fluid from leaking into the area above the top of valve rotor 136 within first portion 306.

A thrust bearing assembly 360 is provided at the upper end of valve rotor 136. Thrust bearing assembly 360 includes a thrust washer 362 and a thrust bearing 364. Thrust bearing assembly 360 further includes a wave washer 366 on top of thrust washer 362. Wave washer 366 has an undulating configuration, such that the "waves" apply a downward pressure on thrust washer 362. Thrust bearing 364, in turn, provides a downward pressure on valve rotor 136 and O-rings 332, 338 and 340 such as to provide a leak proof valve. It would be apparent to one skilled in the relevant art that other types of springs or similar devices could be used to pre-load the O-rings such that they are sealed.

B. Port Layout

Referring now to FIG. 4, a schematic sectional view taken along a line 4-4 of FIG. 2 is shown. In particular, FIG. 4 shows a top view of upper surface 314 of second portion 308, on which is shown an example of the placement of oligonucleotide synthesis reagents about inlet ports 146 of rotary valve assembly 138, according to an embodiment of the present invention. The particular placement of reagents as shown in FIG. 4 allows the solid phase synthesis to

yield oligonucleotides of high purity and yield. As will be recognized, the number and placement of reagents may vary depending on the use of the invention. For example, if used in peptide synthesis or nucleic acid or protein purification, the configuration of the port layout can be determined based on the type and sequence of the reaction.

Starting from a home position 404 and working clockwise, four tetrazole (TET 1, TET 2, TET 3 and TET 4) positions 408, 420, 432 and 444, respectively, are spaced apart such that they flank the phosphoramidite (A, G, C, T) positions 412, 416, 424 and 428, respectively, and further flank three additional (X, Y, Z) positions 436, 440 and 448, respectively. The X, Y and Z positions are provided to allow for a variety of modifications of the bases. For example, positions X, Y and Z could be used for mixed bases, labeled nucleotides, nucleotide derivatives, nucleotides to which reporter molecules are linked, or spacers, such as carbon or polyethylene glycol spacers. Positions X, Y, and Z could also be used for deoxyuracil, deoxyinosine, phosphorothiates, and other 5' modifications, such as phosphorylation, biotin, fluorescein, rhodamine, primary amine or fluorescent dyes, available from Life Technologies, Inc., Rockville, MD. Still further, the X, Y, and Z positions could be used for 5' modifiers (e.g., Amino-Modifier C3, C6, C12, Amino-Modifier 5, Amino-Modifier C6 TFA, Phosphorylation, or Thiol-Modifier C6), 5' or 3' modifiers (e.g., Thiol-Modifier C6 S-S, Spacer 9, Spacer C3), sequence modifiers (e.g., Amino-Modifier C6 dT, Amino-Modifier C2 dT, Biotin-dT, or Carboxy-dT), labeling reagents (e.g., Psoralen C2, Biotin, Fluorescein, Acridine, BioTEG, Cholesteryl-TEG or DNP-TEG), or 3' modifiers (e.g., Amino-Modifier C3 CPG, Amino-Modifier C7 CPG, Thiol-Modifier C3 S-S CPG, 3'-Phophate CPG, BioTEG CPG, Fluorescein CPG or Acridine CPG), currently available from Glen Research, Sterling, VA.

Tetrazole is a mild acid activator for the phosphoramidites. In use, valve rotor 136 will alternate between the inlet port for the selected phosphoramidite to be added and its adjacent activator port. These two fluids are alternately introduced into passageway 348 of valve rotor 136 in short, timed pulses so that

mixing of the phosphoramidite and the activator occurs in passageway 348 before the fluids reach reaction chamber 160.

Continuing clockwise, ancillary reagents (CAP B, CAP A, OXID 1, OXID 2 and TCA) have been placed at positions 456, 460, 464, 468 and 472, respectively, with 2 flanking acetonitrile (ACN 1 and ACN 2) positions 452 and 476, respectively. The CAP B and CAP A ports are for a 2-part capping reagent used after the coupling phase. Because the capping agent is unstable when mixed, it is stored in two parts. As such, the ports for CAP A and CAP B are located adjacent one another, so that the valve rotor can easily alternate between the two ports. In use, these two fluids are alternately introduced into passageway 348 of valve rotor 136 in short, timed pulses so that mixing of CAP A and CAP B occurs in passageway 348 before the fluids reach reaction chamber 160.

Position 464 is a port for OXID 1, the oxidation reagent, which is used at the end of each addition of a phosphoramidite, after the capping phase. Position 468, labeled OXID 2, can be another port for an oxidation reagent, or it can be used as a spare port for another type of reagent, such as, a sulfurizing reagent. TCA, the deblocking or detritylation reagent, is positioned at position 472, close to the home position 404, because the detritylation phase occurs just before each new phosphoramidite is added. The locations of ACN 1 and ACN 2 at positions 452 and 476 allow for fast purging, when necessary, of reagents with ACN as it is used in the synthesis cycle.

Finally, GAS is provided at a position 480, adjacent to the home position 404. Gas can be any gas, preferably an inert gas, more preferably a gas from the group consisting of helium, nitrogen or argon. Gas from position 480 is used to evacuate reagent from valve rotor 136 and reaction chamber 160 and to dry the solid support in reaction chamber 160. In particular, by drying the solid support between different steps in the synthesis process, the solid support is better able to accept the next fluid that is introduced into reaction chamber 160. When using CPG as the solid support, for example, drying of the CPG between steps will allow the CPG to make use of the capillary action in the pores of the CPG to better accept the next fluid introduced into the reaction chamber 160. Further,

by using gas to evacuate the system, the contamination ratio is reduced, because the dilution of the reagents due to undesired mixing of reagents is reduced.

In an example of the present invention, the steps for the addition of a base in the β-cyanoethyl phosphoramidite synthesis cycle is provided. Detritylation is generally performed as the first phase of the synthesis cycle. Valve rotor 136 starts at the home position 404. Valve rotor 136 then rotates counterclockwise to the deblocking or detritylation reagent (TCA) position 472. From position 472, TCA is dispensed into reaction chamber160 via valve rotor 136 in several, preferably nine, timed pulses with predetermined pauses between each pulse.

Valve rotor 136 then rotates clockwise to the adjacent wash (ACN 2) position 476 and dispenses one timed pulse of ACN before rotating again clockwise to the gas dispense (GAS) position 480 and dispensing gas. As discussed above, the gas is used at this stage to flush the reagents from the support surface, as needed, and to dry the support surface.

In a second phase of the synthesis process, i.e., the coupling phase, the base is added. In this example, the phosphoramidite adenine is added. Valve rotor 136 rotates clockwise to the TET 1 position 408 and one short timed pulse of the activator tetrazole is dispensed. Valve rotor 136 then rotates clockwise to the A position 412 and one timed short pulse of the phosphoramidite adenine is dispensed. Valve rotor 136 then alternates back and forth between positions 408 and 412, so that alternating pulses of the phosphoramidite and the activator are dispensed into passageway 348 of valve rotor 136. As these fluids travel through passageway 348 and through swirl chamber 350, they are mixed together and then enter the reaction chamber 160.

In a third phase of the synthesis process, i.e., the capping phase, the capping reagent is added. As such, valve rotor 136 rotates counterclockwise to the CAP A position 460 and a short timed pulse of one part of the capping reagent is dispensed. Valve rotor 136 then rotates counterclockwise to the CAP B position 456 and a short timed pulse of the other part of the capping reagent is dispensed. As explained above, since this capping reagent is unstable when mixed, it is distributed in two parts. As such, valve rotor 136 alternates between

positions 460 and 456, so that alternating pulses of the two parts of the capping reagent are dispensed into passageway 348 of valve rotor 136. As these fluids travel through passageway 348 and through swirl chamber 350, they are mixed together and then enter the reaction chamber 160.

In the fourth phase of the synthesis process, i.e., the oxidation phase, the oxidation reagent is added. As such, valve rotor 136 rotates clockwise to the OXID 1 position 464 and a pre-determined amount of oxidation reagent is dispensed. The oxidation reagent adds water molecules to the base that was added to the solid support. Since the other reagents are water sensitive, valve rotor 136 then rotates clockwise to the gas dispense (GAS) position 480 and dispenses gas. As discussed above, the gas is used at this stage to flush the reagents from the support surface, as needed, and to dry the support surface. In one embodiment, valve rotor 136 then returns to the home position 404 and checks to see that the motor drive system registers that it is at home. If there is a failure, and the motor drive system does not register that it is at home, the system will terminate synthesis of this sequence. In another embodiment, valve rotor 136 rotates instead directly to the TCA position 472 to prepare for addition of the next phosphoramidite.

Using the placement of the reagents about upper surface 314 as shown in FIG. 4, the proximity of each of the above reagent locations to each other allows for a shorter cycle time. However, it would be apparent to one skilled in the art of oligonucleotide or peptide synthesis that a variety of positions of the reagents about the inlet ports 146 of valve rotor 136 could be used in the manufacture of oligonucleotides or peptides.

In one embodiment of the present invention, a cycle for the addition of one nucleotide to the growing chain of nucleotides is 1.5 - 3 minutes. Hence, in this embodiment, a 25 base-long oligonucleotide can be synthesized in approximately 38 - 75 minutes according to the present invention. It would be apparent to one skilled in the relevant art that it is possible to achieve cycle times of 30-45 seconds using the present invention, depending on the steps used during the addition of a nucleotide.

A second embodiment of the top view of upper surface 314 of second portion 308 is shown in FIG. 18. In this embodiment, inlet ports 1804 are provided. One inlet port 1808 is connected to a wash solution, such as ACN. A wash channel 1812 is connected to port 1808 and extends between each port. In use, as valve rotor 136 rotates between inlet ports, communicating port 326 of valve rotor 136 passes over the wash solution in wash channel 1812, thereby reducing the contamination ratio.

Further, the upper surface of this alternate embodiment is provided with two outlet ports 1816 and 1820. In the event that fluid collects in the interstitial space between the valve rotor and the second portion of the rotary valve, wash solution can be flushed through the interstitial space. Outlet ports 1816 and 1820 are provided to serve as outlets to drain the fluid from the interstitial space during flushing. In this embodiment, a TEFLON® material, available from E. I. Du Pont de Nemours, may be used for the valve rotor and the stationary portion of the valve rotor assembly, as this material is non-reactive and is rated to be used with the reagents typically used in the synthesis process. It is preferable to use this embodiment with a rotary valve design which does not use O-rings, so that the interstitial space is minimized, as discussed above.

C. Alternate Embodiment

An alternate embodiment of the system of the present invention is shown in FIGs. 21-23. Referring to FIG. 21, a system 2100 is shown. System 2100 includes a distribution plate 2104 and manifold blocks 2108. Distribution plate 2104 and manifold blocks 2108 are designed to be used in place of tubing 140 to connect distribution valves 128 to rotary valves 138.

One problem with using tubing to connect the distribution valves to the inlet ports of the rotary valve is that the tubing is cumbersome when performing multiple synthesis processes, as in the case of a system designed to synthesize 96 oligonucleotides at the same time. Further, the use of large quantities of tubing increases the likelihood that one or more leaks may develop. As such, using tubing for connections is time consuming and requires constant maintenance. Further, because the tubing is made of a somewhat resilient material, the tubing tends to act as a spring. When the distribution valve is on, the flow of pressurized fluid through the tubing causes the tubing to expand slightly. When the distribution valve is then turned off, the tubing returns to its original size, causing extra fluid in the tubing to squirt out of the end of the tubing into the inlet port. System 2100 reduces the likelihood of leakage of fluid from the distribution valve to the rotary valve, and it allows for better control over the exact volume of fluid delivered to the reaction chamber.

As shown in FIGs. 21-23, distribution plate 2104 has inlet ports 2112. Distribution valves 128 are connected directly to inlet ports 2112 by screwing or other attachment means apparent to one skilled in the art. Motor drive systems 142 are attached via bolts, screws or the like to the bottom of distribution plate 2104. Shafts 152 of each motor drive system 142 extends up through holes 2116 in distribution plate 2104 and through aligned holes 2120 in manifold blocks 2108. In one embodiment, distribution plate 2104 and manifold blocks 2108 are made using a TEFLON® material, available from E. I. Du Pont de Nemours. In an alternate embodiment, distribution plate 2104 and manifold blocks 2108 are made from Nickel-coated stainless steel, in which the coating has been applied

using a diffusion bonding process, available from Refrac Systems, Phoenix, Arizona.

Manifold blocks 2108 are attached to distribution plate 2104 using bolts 2132. It would be apparent to one skilled in the relevant art that manifold blocks 2108 could also be attached using screws or some other similar fastening mechanism. Manifold blocks 2108 are composed of layers of material (shown in detail in FIG. 23), each layer corresponding to its own reagent. A top layer 2124 of each manifold block 2108 replaces second portion 308 of rotary valve 138 (as shown in FIG. 3). As shown, inlet ports 146 are formed in top layer 2124. As discussed above, O-rings (not shown) can be placed around inlet ports 146 to reduce the contamination ratio.

As shown in FIG. 22, rotary valves 138 are disposed on top of top layer 2124 of manifold blocks 2108. In particular, valve rotor 136 is attached to and centered on shaft 152 of motor drive system 142. First portion 306 of rotary valve 138 is then placed around valve rotor 136 and is attached to top layer 2124 by screws, bolts or the like. Reaction chamber 160 is disposed on top of rotary valve 138.

In use the fluid or gas enters distribution plate 2104 via inlet ports 2112. The fluid or gas is then distributed to each manifold block 2108 disposed on distribution plate 2104 via channels (not shown) that connect inlet ports 2112 to outlet ports 2128 on distribution plate 2104. In one embodiment, O-rings (not shown), similar to O-rings 332, 338 and 340 discussed above, are seated around outlet ports 2128 to fit between distribution plate 2104 and manifold blocks 2108. The O-rings allow for leak proof transfer of fluids from distribution plate 2104 to manifold blocks 2108.

Once the fluid or gas enters manifold block 2108, each fluid or gas has a separate pathway, so that no two fluids or gases touch or interact. As shown in FIG. 22, the fluids or gases first travel up a vertical channel 2204 in manifold block 2108 to reach the layer of the block dedicated to that particular fluid or gas. An example of one layer 2308 of manifold block 2108 is shown in FIG. 23.

Once the fluid or gas reaches layer 2308 in manifold block 2108, the fluid or gas flows around the perimeter of layer 2308 in a horizontal channel 2304. Horizontal channel 2304 is fluidly connected to another vertical channel 2312 that is connected to a particular inlet port 146 in top layer 2124 (shown in FIG. 21). Each inlet port 146 has a corresponding vertical channel 2312 that travels through manifold block 2108. Care must be taken when designing the horizontal channel 2304 in each layer, not to overlap with vertical channels 2312 or 2204 so that contamination of the reagents does not occur. In one embodiment, horizontal channel 2304 in a first layer of manifold block 2108 is placed about the outermost periphery of the layer, as shown in FIG. 23. In each successive layer, the horizontal channel is placed slightly inside the horizontal channel in the layer below it so that no overlap occurs. Each fluid or gas has a separate layer 2308. There can be numerous layers 2308, depending on the number of fluids or gases to be supplied to inlet ports 146.

In the embodiment shown in FIGs. 21-23, manifold block 2108 is designed to accommodate 8 rotary valves and motors, and distribution plate 2104 is designed to accommodate 6 manifold blocks 2108 or 48 rotary valves. As would be apparent to one skilled in the relevant art, distribution plate 2104 can be designed to accommodate any number of rotary valves. In one embodiment, two distribution plates 2104 are placed adjacent one another, so that synthesis of 96 rotary valves can occur simultaneously.

III. Motor Drive System

Referring to FIG. 5, motor drive system 142 is shown. As discussed above in Section **II**, motor drive system 142 is used to rotate valve rotor 136 to its commanded positions.

In one embodiment, as stepper motor 144 is used to rotate valve rotor 136. A stepper motor is attractive for use in this application, because it may be used as a direct controller, without resort to gear trains. In one embodiment, stepper motor 144 has 200 steps per rotation.

Although stepper motors are inherently simple devices, they still require external means of determining a zero position or "home" reference. Stepper motors usually have four windings which must be energized either in pairs or singly as the motor armature is rotated. The unique excitation pattern for each step must be repeated every four steps as the motor is rotated. The rotational position of the motor is monitored by keeping track of the number of steps, and their directions.

Usually, the driver electronics for the motors are supplied as either computer compatible cards that enable the operation of three or four motors or a separate system for determining a zero reference for the motors.

In the present embodiment, driver electronics 504 and a zero position reference system 508 are mounted on a rear end of stepper motor 144, as shown in FIG. 5, and are used in place of the conventional computer cards or zero reference systems of conventional motors. In particular, driver electronics 504 are mounted on a circuit board 506. In one embodiment, motor drive system 142 is controlled directly from a host computer using only five binary bits of data. Four bits are used for motor excitation and one bit for reporting back a zero reference position. With this motor drive system 142, one can easily operate over 100 stepper motors simultaneously by means of digital input/output (IO) cards mounted in the host computer. In one embodiment, optical isolation of the motor circuits of driver electronics 504 may be preferable to reduce the susceptibility that the computer's electronics will be damaged by the high voltage transients and other external forces normally associated with driving high-power inductive loads. In one embodiment, the optical isolation is located on a separate circuit board from driver electronics 504.

With currently available computers, such as a PC, it is possible to generate a motor pulse excitation pattern for multiple motors directly by the host computer. In one embodiment, between 1-96 stepper motors or more, one for each rotary valve assembly, are simultaneously operated by means of a single Pentium PC computer operating at 233 MHZ.

Motor drive system 142 of the present invention uses a stepper motor 144 having shaft 152 available at both ends of stepper motor 144. A first end 512 of shaft 152 is used for attaching stepper motor 144 to valve rotor 136. First end 512 may be coupled directly to valve rotor 136, or, as shown in FIG. 5, first end 512 may be coupled to an extension shaft 516, which is then coupled to valve rotor 136. A second end 520 is used for mounting zero position reference system 508, as discussed in further detail below. It would be apparent to one skilled in the relevant art that a stepper motor having a shaft available at only one end of the motor could also be used, where driver electronics 504 and zero position reference system 508 are attached to the same end of the shaft as the valve rotor 136.

Driver electronics 504 are connected to a host computer (not shown) via a connector 548 and preferably through the optical isolators described above. Driver electronics 504 control stepper motor 144 such that the position of stepper motor 144 is monitored and controlled by the host computer sending Transistor-Transistor Logic (TTL) level signals to driver electronics 504. This makes it possible to include zero position reference system 508 along with driver electronics 504 as part of motor drive system 142 by taking advantage of the ability of the host computer to generate motor excitation signals, keep track of motor positions, and read a position sensor.

A. Zero Position Reference System

Zero position reference system 508 consists of a Light Emitting Diode (LED) 524 which is mounted on a fixed plate 528. A rotating aperture disk 532 is mounted on second end 520 of shaft 152. A silicon detector 536 and an operational amplifier (OA) 540 are fixedly mounted on a circuit board with driver electronics 504.

To accurately identify a unique motor position out of 200 positions, it is essential that zero position reference system 508 be accurate to approximately one part out of 400. Silicon detector 536 has a rectangular sensing element (not shown). In one embodiment, the rectangular sensing element is approximately

0.008 inches by 0.240 inches. Aperture disk 532 has a radial aperture 544 having a width matching the size of the sensing element of detector 536. As such, in the above-described embodiment, the width of radial aperture 544 is approximately 0.008 inches.

It would be apparent to one skilled in the relevant art that one or more detectors 536 could be used to detect more than one motor position. For example, additional radial apertures and detectors could be added to the aperture disk, such that the zero position reference system 508 could identify when the motor is at a "home" position and at a midway position.

Aperture disk 532 rotates in close proximity to the sensing element of silicon detector 536, and source LED 524 is mounted below aperture disk 532. In one embodiment, LED 524 is mounted approximately 0.5 inches below aperture disk 532. By incorporating the narrow sensing element mounted with its long axis radial to shaft 152, a maximum signal is available when aperture 544 of aperture disk 532 uncovers the light from LED 524.

Operational amplifier 540 is connected to silicon detector 536 and is used to amplify the signal from detector 536 so that it can be sent back to the host computer. In one embodiment, operational amplifier 540 is a Micropower Single-Supply Rail-to-Rail amplifier (OP191). This particular amplifier is preferable because it can use the same power supply as used to run stepper motor 144. As such, in this embodiment, zero position reference system 508 is powered by the same 5 to 12 volt supply that provides the excitation power to stepper motor 144. Further, because motor drive system 142 can make the position measurement using zero position reference system 508 after shaft 152 of stepper motor 144 has ceased its rotation, there is less electrical interference due to the motor excitations.

B. Driver Electronics

Driver electronics 504 are provided to develop the pulses used to drive the motor and to ramp up the step speed, when necessary. The host computer sends excitation pattern signals to the driver electronics to indicate where the

motor should go (i.e., one step clockwise or one step counterclockwise) and updates the position information of the motor. In one embodiment, stepper motor 144 is provided with 20 different positions, each position being noted with an increment of 10 steps, (e.g., the first position, usually the "home" position, is 200, the second position is 210, and so on up to 390). Each position corresponds to an inlet valve 146 of rotary valve assembly 138.

In one embodiment, driver electronics 504 consist of four power Darlington transistors along with diode clamps to protect the power transistors from reverse voltage transients when the transistors are turned off. The excitation pulse widths and timing are determined directly by the host computer thus minimizing the amount of electronics that has to be mounted on stepper motor 144 to make them computer compatible.

Each stepper motor 144 is connected to a host computer by means of external optical isolation boards and digital IO cards mounted in the computer. In one embodiment, six 96 bit IO cards are used to control 96 stepper motors.

In one embodiment, an antibacklash feature is also incorporated into the computer program that drives stepper motor 144, discussed in further detail below, such that the rotation of the valve rotor 136 always finishes in the same direction independent of its initial direction of rotation. For example, the computer program is designed such that the stepper motor 144 causes valve rotor 136 to rotate one step past its desired location and then come back one step. This antibacklash feature is preferable to overcome any drag to the rotation of valve rotor 136 due to O-rings 332, 338 and 340

IV. Output Monitor

Referring to FIG. 6, an output monitor 600 is shown. Output monitor 600 is a Dual Wavelength Spectrophotometer that is used in conjunction with synthesis system 100 (shown in FIG. 1) to monitor the efficiency of the reactions being performed by system 100. In one embodiment, output monitor 600 monitors the amount of Trityl present in the effluent for an oligonucleotide

synthesis process. Output monitor 600 can also be used to monitor the amount of the various reagents or chemicals present in the effluent from a reaction.

As is well known in the art. during synthesis, a critical measurement of efficiency of the process is the amount of Trityl present in the effluent. Use of a monitoring agent, such as trityl-based groups, to monitor yield is described in further detail in U.S. Patent No. 4,800,166 to Horn *et al.*, which is incorporated herein by reference.

The nature of the Trityl spectrum is such that the absorption of essentially blue light, as may be provided by a blue Light Emitting Diode (LED), is functionally related to the amount of Trityl present in the effluent. In the present invention, the Trityl monitoring process is carried out while the Trityl containing effluent is inside tubing 140 normally used in the manufacturing process.

Another measure of efficiency of the synthesis process is the measure of the amount of reagent or chemical in the effluent. If there is a large increase in the amount of a particular reagent or chemical in the effluent, this is an indicator that the reaction with that reagent or chemical is complete. This type of feedback can be used to optimize the amount of reagent or chemical delivered to the reaction chamber to insure that a complete reaction has occurred and to reduce waste of reagents or chemicals.

The Dual Wavelength Spectrophotometer of output monitor 600 consists of an optical sensor 604, LED drivers 608, and a signal processor 612.

A. Optical Sensor

Referring to FIGs. 6 and 7, optical sensor 604 is made in two parts with a common circular cross section path between the two parts that provides a space for the location of tubing 140. In a first part, a blue LED 616 and a red LED 620 are mounted adjacent to each other in a row along one side of tubing 140. The light emanating from LEDs 616 and 620 passes through a narrow slit 704 (shown in FIG. 7) that is parallel to and centered on an axis 708 of tubing 140. The narrow width 706 of slit 704 is chosen to match the internal diameter 710 of

tubing 140. The length of slit 704 is consistent with the combined diameters of the two LEDs 616 and 620.

A detector assembly 624, which includes a silicon diode optical detector 628 with an internal amplifier (not shown), is located behind a comparable slit 712 in a second part of optical sensor 604, directly opposite illumination slit 704.

LEDs 616 and 620 are turned on alternatively at a 1KHz rate. The optical light path between LEDs 616 and 620 and detector assembly 624 is designed using aligned slits 704 and 712 and the position of LEDs 616 and 620 to optimize the transmission of LED light through the center of tubing 140 and minimize the transmission of light around the effluent by means of the sidewalls of tubing 140.

B. LED Drivers

LED Driver 608 consists of an analog square wave oscillator which is counted down and decoded to provide a series of signals. First, a low source impedance 1 KHz square wave signal capable of driving up to 96 LEDs simultaneously is provided.

LEDs 616 and 620 are connected in parallel with reversed polarity so that they are alternately turned on during a half cycle of the square wave. Variable series resistors for each diode are included so that the light outputs can be matched to a standard value. Since it is also desirable to monitor the resultant light transmission signals only during the second half of each illumination period, a set of measurement gates are derived by decoding the base oscillator signal and the resultant divided down square wave.

C. Signal Processor

Referring to FIG. 6, the signal from silicon detector 628 is amplified by an internal amplifier (not shown) to a value of plus ten volts for both the red and blue emissions. The matched values are achieved using a clear effluent and by adjusting the individual light levels by means of their series potentiometers.

In the example of a Trityl measurement, as the Trityl concentration increases, the blue signal will be attenuated and the red signal will remain

essentially constant. These two signals are separated using a synchronous demodulator and filter 632, consisting of two analog switches (not shown) and a differential amplifier (not shown).

The differential output is then demodulated and filtered with a low pass filter (10Hz cutoff) 632, and passed to an Analog-to-Digital converter card 636 mounted in a digital computer 640. The signal that is passed to Analog-to-Digital converter card 636 is a nonlinear function of the Trityl concentration. This would also be true even if the measurements were made in a quartz cuvette where a simple exponential function would suffice for linearization.

In one embodiment, the measurement is complicated by the presence of a stray light path around the Trityl solution in the wall of tubing 140. Consequently, the data is linearized using an empirical equation developed by correcting a voltage versus concentration curve plotted by means of an EXCEL spreadsheet. This algorithm is satisfactory even with multiple channels.

In the described system, reference measurements are made using red LED 620. By subtracting the red signal, it is possible to eliminate uncorrelated noise and sensor drift.

In the Trityl measurement example, the measurement can be displayed by computer 640 in numerical and/or graphical form to the user so that continuous monitoring of the efficiency of the synthesis process can be achieved. Further, adjustments to the synthesis process, based on the Trityl measurements, can be made during the process to increase efficiency and reduce unnecessary waste of the reagents.

In one embodiment, the measured signals from the output monitor exhibit a shift in the assumed base line reference when going from one clear reagent to another. For example, a marked shift would be seen in the monitor output voltage when ACN was substituted for TCA even though the color of both reagents appeared to be colorless and the same.

When the same measurements were taken across a rectangular quartz cuvette instead of making the measurements across the tubing (having a circular

cross section), the measured values and the baseline across the square cuvette using TCA or ACN were identical.

An example of the output measurements for the different reagents in the effluent from an oligonucleotide synthesis process using system 100 of the present invention is shown in FIG. 19. In particular, FIG. 19 shows a graph having a curve 1904. Curve 1904 is a representative curve of the effluent during the addition of a phosphoramidite. Curve 1904 has several peaks that represent the different segments of the synthesis process. The first peak 1908 represents the trityl in the effluent. The second peak 1912 represents the coupling segment, in which an additional base is added. The third peak 1916 represents the capping segment, in which the capping reagent is added to the reaction chamber. The fourth peak 1920 represents the oxidation segment, in which the oxidation reagent is added to the reaction chamber.

Also shown in FIG. 19 is the shift in the baseline reference when a new reagent is introduced into the reaction chamber. For example, a baseline for the wash solution (ACN) used before the detritylation segment is shown at a baseline 1924. A lower baseline for the detritylation reagent (TCA) is shown at a baseline 1928. Once the reaction is complete, more of the reagent is present in the effluent leaving the reaction chamber, thereby causing a baseline shift. As such, continuous monitoring of the effluent will indicate when a reaction with a particular reagent or chemical is complete. If a reagent or chemical is still being applied to the reaction chamber after the reaction is complete, then the time interval for that reagent or chemical should be adjusted to avoid waste. Similarly, if the supply of a reagent or chemical is cut-off during the reaction, the time interval for that reagent or chemical should be adjusted to ensure a complete reaction has occurred.

One theory for why the output voltage changes for different reagents, when taking the measurement across the tubing, as described in the present invention, is that the measurement vessel, i.e. tubing, has a circular, lens-like shape, and the reagents have different indices of refraction. Changes in the index of refraction resulting from changes in the reagents would result in a

corresponding change in the effective focal length of the cylindrical lens formed by the inside bore of the tubing. These changes in focal length could easily alter the distribution of light transmitted from the LEDs to the detector, which would result in a change in the measured baseline, as shown in FIG. 19.

In the present invention, the change in the measured baseline for different reagents allows the output monitor to detect the washout characteristics of the system as various reagents are passed through the reaction chamber. Typically, Trityl monitors measure only the Trityl output during a wash (ACN) cycle. The advantage of the output monitor of the present invention, is that it can detect how much of the effluent leaving the reaction chamber is wasted reagent. Since the reagents used in synthesis are expensive, it is important to use only as much reagent as is necessary for the synthesis process.

In another embodiment, the reagents are colored with different colors and/or dyes or with different concentrations of the same color and/or dye, depending on the number of LEDs used. As such, each reagent flowing through the tubing will absorb the light from the LEDs differently. In this way, the output monitor can be used to specifically identify which reagent is being added to the sequence at any given time. This output can be used to obtain a profile of the end product to confirm that the correct reagents were added in the correct order.

By using the feedback of the output monitor of the present invention, the amount of reagent or chemical applied to the reaction chamber during a particular reaction can be adjusted to reduce waste of the reagents or chemicals. For example, the results from the output monitor can be fed back into the host computer to adjust the timing of the event or the pressure of the system during addition of the reagents or chemicals to control the amount of reagents or chemicals being delivered to the reaction chamber. This will optimize the use of the reagents and chemicals to avoid waste and ensure a complete reaction.

In an alternate embodiment, two or more separate output monitors may be used. For example, a first output monitor for measuring the amount of a particular reagent in the effluent is disposed directly adjacent the outlet from

reaction chamber 160. This placement allows for almost real-time feedback of the data to the host computer for optimization of the synthesis process. A second output monitor for measuring the trityl concentration is disposed a specified distance away from the outlet of reaction chamber 160. This placement away from the reaction chamber is preferable for the trityl measurement because in detritylation small pulses of the reagent TCA are fed through reaction chamber 160, with small pauses between each pulse. Small pulses are used to better control the volume of TCA used for detritylation. For performing the integration over time necessary to calculate efficiency as discussed in detail below, a steady flow of liquid through the tubing at the site of the trityl monitoring is preferable. As such, a long, steady push of a wash solution follows the detritylation segment, to push the trityl past the output monitor at a constant velocity.

V. Level Sensor System

In one embodiment of the invention, storage vessels 120 and 124 may be equipped with a level sensor system 1700, as shown in FIG. 17, that indicates to the host computer when one or more of the storage vessels is empty or close to being empty. A cross sectional view of one of storage vessels 120, 124 is shown in FIG. 17, such that the sidewalls 1704 of the vessels are visible. A LED 1708 is placed on one portion of sidewall 1704 such that LED 1708 is located within a curved portion of the sidewall. A silicon detector 1712 is positioned opposite LED 1708 on the opposite curved portion of sidewall 1704, as shown in FIG. 17.

LED 1708 is powered with a 5 volt power source. Both LED 1708 and silicon detector 1712 are connected to ground, as shown in FIG. 17. Further, output from silicon detector 1712 is sent to an analog-to-digital converter (not shown) and then sent to the host computer of system 100.

LED 1708 and detector 1712 are placed near the bottom of the periphery of the storage vessel. When there is liquid in the storage vessel, the light from LED 1708 travels directly across the storage vessel to silicon detector 1712 as shown in FIG. 17 by the dashed lines with arrows. However, when there is no liquid above the light from LED 1708, the light is refracted because the index of

refraction of the liquid is different from the index of refraction of air (e.g., no liquid). In this case much less of the light from LED 1708 reaches silicon detector 1712. As such, an output signal is sent from silicon detector 1712 to the host computer that one of the storage vessels is empty or near empty.

In one embodiment, this causes the host computer to signal an alarm to the user to refill the particular storage vessel which is empty. However, in another embodiment, each of the smaller storage vessels are fluidly connected to corresponding larger stock bottle. When the host computer receives a signal that a particular storage vessel is empty or near empty, the host computer may send a signal to cause automatic refill of the smaller storage vessel from a stock bottle. In one embodiment, the signal is sent to a valve or pump that causes the automatic refilling of the storage vessel.

Many of the reagents used in the synthesis process degrade over time. As such, it is preferable to use all the older reagent in the smaller vessel before refilling. Further, automatic refilling is preferable because the reagents are typically all clear liquids, and it is easy for human error to occur, wherein a storage vessel is accidentally refilled with the wrong reagent or chemical. In the embodiment in which the vessels are refilled automatically by the system, the only human involvement, and thus the only chance for human error, is in the initial connection of the larger stock bottle to the smaller storage vessel. After that point, all refilling occurs without human intervention.

VI. Process of the Invention

System 100 controls the reactions via a set of rotary valves and distribution valves which modulate the flow of a plurality of fluids or gases. Any given fluid or gas is selected by the rotary valve, and a volume to be dispensed is measured by activating a distribution valve for a specified time. This time period is determined based on the volume of fluid or gas to be delivered to the reaction chamber, and the pressure of the fluid in the fluid vessel or the pressure of the gas.

Further, calculation of the time period for activating a distribution valve takes into account the number of channels, i.e., reaction chambers, being supplied at one time. If many channels are being supplied by the distribution valve, then it will take longer for the fluid or gas to reach all of the inlet ports. As such, the amount of flow is modified by the number of channels being supplied. In one embodiment, a look-up table is created which will tell the computer what the time period should be based on the number of channels being supplied.

Coordination of all valves (distribution and rotary) to accomplish simultaneous actuation and hence simultaneous reactions in all reaction chambers is done by inputting a sequence into a sequence table and using an event matrix table to convert the sequence into a series of instructions for the host computer. In one embodiment, these instructions are generated as a comma delimited file by a Microsoft® Excel spreadsheet. An example of a series of sequence tables are shown in FIGs. 8A, 8B and 8C. An example of an event matrix is shown in FIG. 9.

A. Sequence Table

Referring to FIGs. 8A-8C, a desired sequence for an exemplary oligonucleotide synthesis is inserted into a table 800, in the spaced provided in a row 804. In one embodiment, the sequences in table 800 are provided by the customer in the form of a computer file that is imported into the host computer. In the embodiment shown in FIG. 8A, there are eight rows 804. Each row 804 is numbered to its left with its corresponding channel number. Each channel number corresponds to a different sequence being made. In this example, there are eight different sequences that may be input in table 800. In another embodiment, 96 or more different sequences can be entered at once.

In addition to entering the desired sequence, the user must also indicate in table 800 whether the end base in the sequence should be subjected to detritylation. If detritylation occurs, this allows additional bases to be added on the sequence in a subsequent synthesis. If detritylation does not occur, then the

resulting sequence is not open to further synthesis. As such, as shown in FIG. 8A, the terms "DMT" or "NO DMT" are used to indicate whether detritylation or no detritylation will occur at the end of the synthesis process.

The sequences are then converted, as shown in a table 808. The first conversion merely reverses the order of the items in the sequence so that they appear in the order in which they are delivered to the reaction chamber. For example, in the first row 804 of table 800, the sequence is "G A C C T G T C A G". In a corresponding first row 812 of table 808, the sequence has been reversed to read "G A C T G T C C A G." Further, in this first conversion, the DMT instructions are assigned a number. In this example, the number 4 is used to indicate an instruction of detritylation or DMT. The number 5 is used to indicate an instruction of no detritylation or NO DMT. This number is added directly after the re-ordered sequence.

In this example, there are forty locations provided for input of a sequence. After input of the sequence and the detritylation instruction, the remaining locations in the table are filled with the letter S for "space". In one embodiment, those locations marked with the letter S are filled with instructions for the valve rotor to go the home position.

Table 808 is converted again so that the instructions are presented in a format readable by the host computer. This conversion is shown in table 816 in FIG. 8C. In table 816 each instruction from table 808 is converted into a number by reference to an event matrix 904, as discussed below with reference to FIG. 9.

B. Event Matrix

As shown in FIG. 9, the steps for adding each base during the exemplary oligonucleotide synthesis process is shown in event matrix 904 as a series of events. In one embodiment, event matrix 904 is provided using a Microsoft® Excel spreadsheet. The rows 908 indicate each event in the event matrix. In one embodiment, there are a total of 54 events or rows. Although the event matrix is discussed with respect to oligonucleotide synthesis, it would be apparent that

the event matrix can be adapted for use in a variety of reactions requiring delivery of a sequence of fluids and/or gases.

A column 908 lists the event numbers for each event. An adjacent column 912 lists the volume in milliliters of each fluid or gas delivered during a particular event. This volume measurement is based on the pressure in the fluid vessel or the pressure of the gas and the time interval during which the distribution valve corresponding to the particular fluid or gas is open. This time interval for each event is shown in a column 916 in milliseconds. If no fluid or gas is delivered during a certain event, the corresponding row in column 912 will indicate a "WAIT" event and the corresponding distribution valve position, as described in detail below, will indicate an "OFF" position.

Columns 920 and 924 show instructions for a Home or "H" position. Column 920 relates to the instructions sent to valve rotor 136. As shown in FIG. 9, if the Home position is selected, valve rotor 136 will receive the instruction to return to the "Home" position. Column 924 relates to the instructions sent to distribution valve 128. If the home position is selected, then there will be no fluid or gas flowing to valve rotor 136. As such, distribution valve 128 will receive the instruction to remain in the "off" state.

The following columns 924, 928, 932, 936, 940, 944, 948, 952, 956, 960, 964, 968, 972, 976 and 980 indicate a rotary valve position and a corresponding solenoid valve state for each event necessary to add a particular phosphoramidite to a sequence. For example, column 924 lists the rotary valve positions for each event used to add adenine to the sequence being synthesized. Column 928 lists the request of states of distribution valves 128 for each event according to the needs of a particular phosphoramidite cycle. In the case where more than one sequence is being synthesized at the same time, each channel corresponding to a sequence sends its list of events from a particular column to the processor, and the computer program combines the events by eliminating duplicates such that the proper set of distribution valves are energized.

When the event matrix is developed, the various rotary valve positions are described by the corresponding reagent mnemonic, such as TCA or ACN1,

etc. Some reagents may be available at two or more rotary valve positions. In this case, a numerical suffix is employed to designate the different locations.

As discussed above, a particular reagent vessel may be providing reagent to more than one inlet port of the rotary valve assembly. In this case, all the inlet ports for that reagent are controlled by a single distribution valve. As such, as shown in row 1 of column 928, the rotary valve instructions tell the rotary valve to travel to the ACN2 position. However, the distribution valve state indicates only an ACN state, because there is only one state for ACN on the distribution valve.

Columns 984 and 988 indicate a rotary valve position and solenoid valve state for the detritylation step or DMT. As shown in a column 992, event matrix 904 is delimited at its rightmost limit with an "R" for return and at its bottom limit with a "B" for bottom, to allow automatic interpretation of this file by the DNA synthesis software. Modification of the synthesis cycle is then carried out in the Excel spreadsheet which then generates a comma delimited event matrix file.

Each instructional column in event matrix 904 (i.e., columns 916 - 988) are given an ascending column number, beginning with one and ending with nineteen, as shown in FIG. 9. These column numbers are used to convert chart 808 to the chart shown in FIG. 8C.

Referring now back to the example in FIG. 8C, each item in the sequence is replaced with the corresponding rotary valve column number for that particular item. For example, the first base in row 812 of table 808 is "G". The corresponding rotary valve column in event matrix 904 is column 8. As such, the first item in a corresponding row 820 of table 816 is the number "8". Each base is converted into its numerical equivalent.

At the end of the sequence, if there is a "4" indicating detritylation, the "4" is replaced with an "18" corresponding to column 984 on event matrix 904. If there is a "5" at the end of the sequence, indicating no detritrylation, the "5" is replaced with a "2" corresponding to column 920, which instructs the rotary valve to return to the "Home" position. Similarly, all of the "S" instructions in

table 808 are replaced with a "2" in table 816 to indicate the "Home" position. The instructions in table 816 are then sent to the host computer which uses these instructions to control the motor drive system 142 and distribution valves 128 during the synthesis process.

VII. Method of Performing A Reaction Using the Invention

The host computer that controls the reaction is programmed with a computer program to run the process. In one embodiment, the computer program is written in Microsoft® Visual Basic operating under Microsoft® Windows 95 or NT. Microsoft® Visual Basic provides for a relatively easy way to obtain user friendly interactive graphics along with the ability to communicate with motors and distribution valves (e.g., solenoid valves) by means of plug-in digital and analog Input/Output cards. It would be apparent to one skilled in the art of computer programming that a variety of programming languages, such as C++ and like programming languages, and a variety of computer systems could be used to implement the method of the present invention.

The computer program uses the event matrix to coordinate the activities on the various channels, each of which may be adding different fluids or gases at the same time. Each channel has its own rotary valve which is used to select the fluids or gases needed to perform the sequence of items for the reaction assigned to that channel. The fluids or gases are supplied to the inlet ports of the rotary valve assembly by means of manifolds which simultaneously supply all of the rotary valves with the particular fluid or gas under their control. For a particular fluid or gas to flow into a reaction chamber, the rotary valve must be in the position dedicated to the fluid or gas and the corresponding distribution valve must be activated.

The data in event matrix 904 is converted by the computer program into a converted table in which the first column contains the time interval in milliseconds assigned to a given row and the second column gives the step location on the stepper motor for a rotary valve position that corresponds to the mnemonic entry. This step location value is expressed as an integer number

between 200 and 400, as described above. This number represents a new position used in the rotary valve control software to command a rotary valve to a new location. In the case of a rotary valve that has twenty inlet port positions, there is a port at each tenth step location (e.g., 210, 220, etc.).

Third and fourth columns are used to contain the state information for the corresponding distribution valve. Two columns are used for this information because two eight bit words (ports) are needed to control sixteen valves. Thus the computer program generates two columns in the converted table from the original distribution valve column in event matrix 904. Digital values are assigned to the desired distribution valve state for each channel and placed in the table. In the example of FIG. 9, the rows represent each event for the addition of a particular base. This converted table fully describes the process steps necessary to add a single base.

FIGs. 10A, 10B and 10C show a flow chart 1000 of the steps used by the computer program to control a synthesis process or reaction using the present invention. The program starts in a step 1002. As shown in a step 1004, system 100 calibrates valve rotors 136 of rotary valve 138 and checks, in a step 1006, to see if valve rotors 136 are in their "Home" positions. If the valve rotors are not in their "Home" positions, a counter is incremented and the system returns to step 1004 to re-calibrate the rotary valves. After three unsuccessful attempts to calibrate the rotary valves, as tested in a step 1008, the process is aborted, as shown in a step 1010 and the process ends, as shown in a step 1012.

If valve rotors 136 are at their "Home" positions, system 100 initializes the monitoring data in a step 1014, initializes the antibacklash flags to zero in a step 1018 and sets the program pause flags to zero in a step 1018. Then, the system is ready to load and convert event matrix 904, as discussed above and as shown in a step 1020, and load and display the desired sequences from table 816, as shown in a step 1022. As discussed above, prior to loading the sequences from table 816, they have been converted from their original form, as shown in table 800.

The original sequence may be manually entered by the user or imported from another computer file, and displayed to the user in a Graphical User Interface (GUI), such as that shown in FIG. 11.

As shown in a GUI 1100 of FIG. 11, a set of Text windows 1104 is provided in which sequences may be entered and/or displayed. In this embodiment, each window is approximately seventy five characters wide, however sequences having up to two hundred items may be entered. It will be apparent to one skilled in the relevant art, that the system may be programmed to accommodate a sequence having any number of items in windows 1104.

At the bottom left side of GUI 1100, a Text window 1108 shows the current Event Matrix Data File that will be automatically loaded in step 1020. This is the Microsoft® EXCEL file mentioned above and for special operations, one might wish to change this default entry to a event matrix data file generated for some other purpose such as PURGE or some form of system testing. The file designator may be edited in the window as necessary.

When the window is full, the sequence will automatically scroll to the left to make room for the additional entries. The sequence may be entered in either upper or lower case letters. Unique and different sequences may be entered in successive windows and the sequences do not have to all be the same length.

System 100 then sets up the analog-to-digital cards for the output monitor and the digital interface cards for the motor drive system and distribution valves to read data, as shown in a step 1024. Further, system 1026 sets up the output monitoring picture boxes for presenting the output data to the user during synthesis, as shown in a step 1026.

After this set-up of system 100 is complete, the computer program is prepared to start the process, as shown in a step 1028. This process is discussed in detail in the flow charts shown in FIGS. 10B and 10C.

The first step in the process is to call the first event, as shown in a step 1030. The system then turns off all of the distribution or solenoid valves in a step 1032 and institutes a 100 ms delay, as shown in a step 1034 before rotating

the rotary valves to their respective rotary valve positions based on the instructions from the event matrix, as shown in a step 1036.

The program communicates to the rotary valves by establishing a new position for each valve rotor in the new position array, as necessary. A sub-routine called "set rotary valves" compares the data in the new position array with the data in a present position array to develop a "Delta" value equal to the difference in the New and Present Positions.

To facilitate going to the new position by the shortest route, a full rotation is added to the stated present position if the difference is greater than one half rotation. If the difference exceeds one half rotation in the opposite direction, one full rotation is subtracted from the stated present position.

As discussed above, stepper motor 144 is employed to move valve rotor 136 from its present position to the desired new position. In one embodiment, stepper motor 144 has four windings and two hundred steps per rotation. The motor windings are energized in pairs with the excitation pattern repeating every four steps. The required excitation pattern (wherein two windings need to be energized) for any particular phase orientation can be identified by performing a Modulo 4 division on the position value. The Modulo 4 remainder is used as an index to a look up table that converts the Modulo 4 remainder to the corresponding excitation pattern.

The communication of the excitation pattern to stepper motor 144 is accomplished by using 4 bits of binary data (one half of an 8 bit digital I/O port). Each 8 bit port controls two motors. Communication to the high bit motors is obtained by multiplying the computed digital value by 16 and adding it to the value for the low bit motor assigned to the same port.

A control algorithm first determines which way the motor must move to advance towards the desired new position provided it is not already at the desired new position. If a motion is necessary, the new position is changed by one step in the desired direction. The program then takes the Modulo 4 remainder for the modified new position and sends the corresponding execution pattern to the motor. This causes the motor to move to the modified new position. This

process is repeated for all of the motors in the system and then a short 5 to 10 ms time delay is executed (motor execution pulse width) to allow the motors to move to their new present position. Since all motors start to move at the same time, the time delay may be adjusted dynamically to improve starting characteristics of the motors.

The system then tests to see if all of the valve rotors have reached their respective destinations, as shown in a step 1038. If one or more of the rotary valves are still moving to a new position, the system goes back through steps 1032-1036 until all of the valves have reached their respective positions. Once the valve rotors have stopped rotating, the system updates the rotary valve position in the computer, as shown in a step 1040. The motor excitations are then all turned off until the next set of valve rotor motions take place. It is at this point that the antibacklash feature, discussed above, is employed to rotate all the motors one step clockwise, followed by rotating one step counterclockwise.

When controlling a stepper motor, there are three possible commands: move one step clockwise, move one step counter clockwise or stay in place. Consequently it is preferable to calibrate the motor to a known position at the start of the program, as discussed in step 1004, and then keep track of how it is oriented as it is directed to go to various new positions, as discussed in step 1040. In the present invention, zero position reference detector 508 is mounted on each motor 144 to identify the "HOME" position. This position is an OFF position for the rotary valve and all other positions are referenced to this location. The "HOME" position is also calibrated to be one of the fifty (Modulo 4 remainder 0) positions that are encountered as the motor makes a full rotation.

At the beginning of the program, all of the motors are rotated one step at a time until the zero position reference sensor 508 establishes that each motor has reached the "HOME" position.

In one embodiment of the present invention, valve rotor 136 has twenty designated positions one of which occur every ten motor steps. Using the above algorithm, 96 stepper motors may be controlled with approximately fifty lines of source code plus the code that is used to set up the various digital I/O card

environment. In one embodiment, this source code is programmed in Microsoft® Visual Basic. However, it would be apparent to one skilled in the relevant art of computer programming that a variety of other computer languages could be used, such as C++ and like programming languages. In another embodiment, approximately thirty lines of additional code are used in the motor position calibration sub routine named rotary valve calibration.

After the rotary valve position has been updated, the appropriate distribution valves are energized based on the state instructions in the converted event matrix, as shown in a step 1042. As mentioned earlier, in one embodiment, the distribution valves are controlled by two eight bit ports. One port gets its assignments from the column in the converted event matrix immediately following the corresponding rotary valve column. The second port gets its assignment from the next column in the converted event matrix (not shown in event matrix 904). Additional distribution valves may be controlled by expanding the number of columns in the converted event matrix.

At the moment when the distribution valves are energized, the system begins the time interval delay for the particular event, based on the time interval information in the event matrix, as shown in a step 1044. Once the time interval delay is completed for the event, as shown in a step 1046, the distribution valves are turned off, as shown in a step 1048.

In one embodiment, a time interval may be set as "Trityl" during a synthesis process. This indicates that during this time interval a Trityl measurement should be made. As such, in a step 1050, the system calls the next event and queries whether the time interval for this event is set as "Trityl". If so, the system takes a Trityl measurement and displays the Trityl data to the user, as shown in a step 1052. It would be apparent to one skilled in the art, that the Trityl data could be shown in a graphical format for each channel performing a synthesis process, or could be displayed in a numerical or other form for the user, an example of which is shown in a GUI 1200, shown in FIG. 12.

Based on the Trityl measurements, the system will calculate and display efficiency data, as shown in a step 1054. In one embodiment, efficiency data is

calculated using the second trityl integral as the reference base. The second trityl measurement is used as a reference base because during the first detritylation, excess trityl is present in the effluent. As such, the first trityl measurement is not an accurate predictor of efficiency. The integral of the present trityl measurement is calculated and compared with the reference base trityl integral to determine efficiency using the following equation.

$$\text{Efficiency} = \sqrt[n1]{\frac{(\text{Present Integral})}{(\text{Reference Integral})}}$$

,where n1 is the number of trityl integrals minus 2. The number 2 is subtracted because the reference integral is based on the second trityl integral.

The system then tests to see whether there is another event in a step 1056. If there are no other events, the program ends in a step 1058. However, if there is another event, the program returns to a point marked X in FIG. 10B, as shown in a step 1060, and follows steps 1032 and so on.

Referring back to FIG. 11, the save Trityl button 1112 opens another GUI 1300, shown in FIG. 13, which provides windows and buttons for saving selected trityl traces to disk files. GUI 1300 has two text windows in which instructions are given for saving Trityl files. Data entered into a Channel Number window 1304 selects the particular channel (i.e., DNA sequence) for which a Trityl trace is to be saved. Data entered into a window Disk File Name window 1308 indicates the file name and location for the above Trityl trace. The file is easily read as an Excel spread sheet. The data includes the analog Trityl trace traces, a corrected Trityl integration for each trace. Time, Date and an Event matrix identifier.

After entering the desired set of sequences, the user may click on the START button 1116 at the bottom center of the screen to initiate the start of the synthesis process. After initializing the system, the screen will change to another GUI in which monitoring screens will be presented.

A STOP button 1120 can be used to terminate the program by setting the event counter and sequence counter to their end values. This sub-routine sets the Rotary Valve in the Home position and the Solenoid Valves in the OFF state.

The RValve Cal button 1124 causes the stepper motors to rotate to their Home positions and stop. The Home position is identified by a zero position reference detector that senses the position of the rotating aperture disk due to light from a LED illuminator and a silicon detector. Once each motor reaches its Home position, the text window 1104 corresponding to each channel turns green. If one of the motors fails to reach the Home position, the text window 1104 corresponding to that motor will remain red.

There are two windows 1132, 1136 located below an RV Set button 1128 in which the Channel Number (Channel) and the Rotary Valve Position (Position) can be set. RV Set button 1128 will then cause the Rotary Valve to go to the requested position. When the requested position is reached, the position window will turn red, unless the requested position is the Home position, in which case position window 1136 will turn green.

There are two windows 1144 and 1148 located below an SV "On" button 1140 in which a Solenoid Valve state can be set manually. SV Valve window 1144 indicates which valve is selected and SV Time window 1148 indicates the time interval for valve activation in milliseconds. If no time is given, the valve will stay activated until an SV "OFF" button 1152 is clicked. The SV Time window 1148 turns green when the valve goes on and remains green during the time interval in which the valve is activated. Once the valve turns off, the SV Time window returns to its normal color.

A "0" Motors "ON" button 1156 causes a motor excitation for the 0 degree phase excitation to the motors. This excitation is used for mechanically setting the aperture disk to one of the 0 degree phase excitations. This excitation will remain on for ten seconds then automatically shut off to avoid motor burn out.

An RV Idle button 1160 can be used to cause the rotary valve to enter an idle condition. Because the O-rings of the rotary valve assembly have a tendency

to stick if the rotary valve remains unmoved for long periods of time, RV Idle button 1160 can be used to cause a pattern of movement of the rotary valve to prevent the O-rings from setting. In one embodiment, the pattern consists of the valve rotor traveling to the Home position, moving one step clockwise from the Home position, returning to the Home position, moving one step counterclockwise from the Home position and then returning to the Home position. This pattern is repeated with the system pausing ten seconds between each step.

A Read Trityl A/D button 1168 is disposed above a Trityl Channel window 1172 and an A/D Value window 1176. The user can set a channel number in Trityl Channel window 1172 and then press Read Trityl A/D button 1168 to get a trityl reading for the selected channel. The raw data for the trityl measurement, i.e., trityl voltage, for the selected channel is displayed in the A/D Value window 1176. This serves as a check to make sure that the output from the output monitor is reaching the host computer successfully. In one embodiment, the output monitor takes readings for ten seconds. These readings are used to adjust the potentiometer on the output monitor circuit boards to calibrate the zero measurement. This is particularly useful when the user changes the tubing in the system, such that the system requires recalibration.

A DecVal button 1180 and a corresponding text window 1184 are shown in FIG. 11. The DecVal button 1180 can be used to cause the system to read the decimal equivalent of the binary value of which motors are in the home position. The results of this test are shown in text window 1184.

An RV test button 1188 is also shown in FIG. 11. This button can be used to test the rotary valves at any time, by manually sending them all to the Home position. As described above, if any of the motors do not reach the Home position, the corresponding text window 1104 will be red. For those motors that reach the Home position, as indicated by the zero position reference sensor, the corresponding text window 1104 will be green.

A Form3 button 1164 calls up another GUI 1200, as shown in a FIG. 12.

GUI 1200 displays a set of Trityl waveform windows 1204, one for each channel. Each window 1204 has two text boxes, one box 1208 for the associated Rotary Valve Position and another box 1212 for the current efficiency calculation for that channel. The efficiency calculation is based on changes in a corrected concentration integration performed on each Trityl trace.

A STOP button 1216 terminates the synthesis and returns the Rotary valves to the Home position with all Solenoid valves turned off. A PAUSE button 1220 sets a software flag that causes the program to pause at the end of the generation of the current base type. The Rotary Valves are sent to the Home position with all Solenoid Valves turned off. A RESUME button 1224 may be used to restart a synthesis that has been interrupted by a pause. The delay time between the pause and resume should not exceed one hour. If the delay is in excess of one hour, the program will automatically terminate as if STOP button 1216 were depressed.

A Form1 button 1228 will return the user back to GUI 1100 where data may be saved or a new DNA Sequence batch may be started.

A Sol A window 1232 displays the current Low Bit Solenoid Valve state as a decimal number. A Sol B window 1236 displays the current High Bit Solenoid Valve state as a decimal number. An Interval window 1240 displays the time interval in milliseconds for the current event.

An Event Length window 1244 displays the number of events in the current event matrix. An Event # window 1248 displays the Event Number of the current event.

A Seq Length window 1252 displays the maximum Sequence Length current used by any of the channels, and a Seq # window 1256 displays the Sequence Number for the current base being generated.

VIII. Environment

The present invention may be implemented using hardware, software or a combination thereof and may be implemented in a computer system or other

processing system. In fact, in one embodiment, the invention is directed toward one or more computer systems capable of carrying out the functionality described herein. An example of a computer system 1400 is shown in FIG. 14. The computer system 1400 includes one or more processors, such as processor 1404. The processor 1404 is connected to a communication infrastructure 1406 (e.g., a communications bus, cross-over bar, or network). Various software embodiments are described in terms of this exemplary computer system. After reading this description, it will become apparent to a person skilled in the relevant art how to implement the invention using other computer systems and/or computer architectures.

Computer system 1400 can include a display interface 1402 that forwards graphics, text, and other data from the communication infrastructure 1406 (or from a frame buffer not shown) for display on the display unit 1430.

Computer system 1400 also includes a main memory 1408, preferably random access memory (RAM), and may also include a secondary memory 1410. The secondary memory 1410 may include, for example, a hard disk drive 1412 and/or a removable storage drive 1414, representing a floppy disk drive, a magnetic tape drive, an optical disk drive, etc. The removable storage drive 1414 reads from and/or writes to a removable storage unit 1418 in a well-known manner. Removable storage unit 1418, represents a floppy disk, magnetic tape, optical disk, etc. which is read by and written to by removable storage drive 1414. As will be appreciated, the removable storage unit 1418 includes a computer usable storage medium having stored therein computer software and/or data.

In alternative embodiments, secondary memory 1410 may include other similar means for allowing computer programs or other instructions to be loaded into computer system 1400. Such means may include, for example, a removable storage unit 1422 and an interface 1420. Examples of such may include a program cartridge and cartridge interface (such as that found in video game devices), a removable memory chip (such as an EPROM, or PROM) and associated socket, and other removable storage units 1422 and interfaces 1420

which allow software and data to be transferred from the removable storage unit 1422 to computer system 1400.

Computer system 1400 may also include a communications interface 1424. Communications interface 1424 allows software and data to be transferred between computer system 1400 and external devices. Examples of communications interface 1424 may include a modem, a network interface (such as an Ethernet card), a communications port, a PCMCIA slot and card, etc. Software and data transferred via communications interface 1424 are in the form of signals 1428 which may be electronic, electromagnetic, optical or other signals capable of being received by communications interface 1424. These signals 1428 are provided to communications interface 1424 via a communications path (i.e., channel) 1426. This channel 1426 carries signals 1428 and may be implemented using wire or cable, fiber optics, a phone line, a cellular phone link, an RF link and other communications channels.

In this document, the terms "computer program medium" and "computer usable medium" are used to generally refer to media such as removable storage drive 1414, a hard disk installed in hard disk drive 1412, and signals 1428. These computer program products are means for providing software to computer system 1400. The invention is directed to such computer program products.

Computer programs (also called computer control logic) are stored in main memory 1408 and/or secondary memory 1410. Computer programs may also be received via communications interface 1424. Such computer programs, when executed, enable the computer system 1400 to perform the features of the present invention as discussed herein. In particular, the computer programs, when executed, enable the processor 1404 to perform the features of the present invention. Accordingly, such computer programs represent controllers of the computer system 1400.

In an embodiment where the invention is implemented using software, the software may be stored in a computer program product and loaded into computer system 1400 using removable storage drive 1414, hard drive 1412 or communications interface 1424. The control logic (software), when executed by

the processor 1404, causes the processor 1404 to perform the functions of the invention as described herein.

In another embodiment, the invention is implemented primarily in hardware using, for example, hardware components such as application specific integrated circuits (ASICs). Implementation of the hardware state machine so as to perform the functions described herein will be apparent to persons skilled in the relevant art(s).

In yet another embodiment, the invention is implemented using a combination of both hardware and software.

IX. Examples

In two examples of the present invention as used for oligonucleotide synthesis, optimization of event matrix 904 was carried out by first synthesizing oligonucleotides on this apparatus followed by cleavage and deprotection of the oligonucleotide product from the solid support. These oligonucleotides were then dried, resuspended in water and quantitated using a spectrophotometer before being analyzed by High Pressure Liquid Chromatography (HPLC). Results are shown in Figures 15A and 15B and 16A and 16B for 17 and 30 base-long oligonucleotides, respectively.

The ideal efficiency of synthesis using the β–cyanoethyl phosphoramidite method is 99.8%. The present invention is capable of producing oligonucleotides with a 99.7-99.8% coupling efficiency. A conventional synthesis apparatus typically results in a lower coupling efficiency, e.g. 98.5-99.5%.

Graphs 1504 and 1604 show the results of the HPLC analysis of the oligonucleotides, where the X-axis is in minutes and the Y-axis is measured in a concentration of absorbance unit, as measured by an ultraviolet (UV) detector. Tables 1508 and 1608 list the quantitative results of the HPLC analysis of the oligonucleotides. Columns 1512 and 1612 show the retention time in minutes for each peak to appear on the UV detector. Columns 1516 and 1616 show the height of each of the peaks on graphs 1504 and 1604, respectively. Columns 1520 and 1620 show the percent area under the peaks on each graph, based on

integrating the area under the curve. Similarly, columns 1524 and 1624 show the percent height of each peak on graphs 1504 and 1604, respectively.

The results are favorable for the oligonucleotides synthesized using the present invention, because the large peak corresponding to the oligonucleotide, shown as peak 1528 on FIG. 15A and as peak 1628 on FIG. 16A, is much larger than the other peaks on the graphs. As shown in table 1508, the percent area under peak 1528 is 95.81% and the percent area under the remaining peaks of FIG. 15A do not exceed 1%. Similarly, the percent area under peak 1628 is 86.16% and the percent area under the remaining peaks of FIG. 16A do not exceed 1.5%.

X. Conclusion

Having now fully described the present invention in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and are herein incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

While various embodiments of the present invention have been described above, it should be understood that they have been presented by way of example, and not limitation. It will be apparent to persons skilled in the relevant art that various changes in form and detail may be made therein without departing from the spirit and scope of the invention. This is especially true in light of technology and terms within the relevant art(s) that may be later developed. Thus the present invention should not be limited by any of the above-described

exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

What Is Claimed Is:

1. A fluid or gas delivery system, comprising:

a rotary valve having a stationary housing with a plurality of inlet ports formed therein and a valve rotor rotatingly disposed in said stationary housing; and

a check valve disposed in said rotary valve.

2. The delivery system of claim 1, wherein said valve rotor has a passageway formed therein such that said check valve is disposed in said passageway.

3. The delivery system of claim 2, wherein said check valve is disposed at one end of said passageway adjacent said inlet ports.

4. The delivery system of claim 2, further comprising:

a swirl chamber disposed at one end of said passageway.

5. The delivery system of claim 4, wherein said swirl chamber comprises a conical shape and is configured to allow mixing of fluids therein.

6. The delivery system of claim 2, wherein said passageway is configured to allow mixing of fluids therein.

7. The delivery system of claim 6, wherein the volume of said passageway is at least twice the volume of the smallest pulse of fluid delivered to said passageway.

8. The delivery system of claim 6, further comprising a reaction chamber disposed in fluid communication with said rotary valve, such that the ratio of the volume of said passageway to the volume of said reaction chamber is within a range of 2:1 to 1:1.

9. The delivery system of claim 1, further comprising:

a motor, wherein said rotary valve is disposed on one end of said motor.

10. The delivery system of claim 9, wherein said motor is a stepper motor.

11. The delivery system of claim 1, wherein said check valve comprises a plurality of check valves, wherein each of said check valves is disposed in one of said plurality of inlet ports.

12. The delivery system of claim 1, further comprising:

a processor, wherein said processor controls rotation of said valve rotor of said rotary valve.

13. The delivery system of claim 1, wherein fluid or gas enters said valve rotor at a lower surface thereof and exits said valve rotor at an upper surface thereof.

14. The delivery system of claim 1, further comprising:

a plurality of distribution valves to control distribution of fluids or gases to said plurality of inlet ports on said rotary valve.

15. The delivery system of claim 14, wherein said plurality of distribution valves are solenoid valves.

16. The delivery system of claim 14, further comprising:

a processor, wherein said processor controls said plurality of distribution valves to allow or restrict the flow of said fluid or gas to said plurality of inlet ports.

17. The delivery system of claim 1, wherein said system is used to perform a synthesis process.

18. The delivery system of claim 1, wherein said system comprises a plurality of rotary valves.

19. The delivery system of claim 18, wherein said plurality of rotary valves comprise between two to ninety-six rotary valves.

20. The delivery system of claim 1, further comprising:

at least one vessel containing said fluid or gas for supplying said system.

21. The delivery system of claim 1, further comprising:

a plurality of vessels each containing a fluid or a gas, wherein said plurality of vessels are fluidly interconnected to said plurality of inlet ports of said rotary valve.

22. The delivery system of claim 1, further comprising:

a pressurized gas source to pressurize said system.

23. The delivery system of claim 1, wherein said fluids are selected from a group consisting of at least one of the following: a nucleotide, a nucleotide derivative, a labeled nucleotide, a wash solution, a capping reagent, an oxidation reagent and a deblocking reagent.

24. The delivery system of claim 1, further comprising:

a monitor for measuring the efficiency of said delivery system.

25. The delivery system of claim 1, further comprising:

a monitor for measuring the quality of the output of said delivery system.

26. The delivery system of claim 1, further comprising:

a graphical user interface, for assisting in control of said delivery system.

27. The delivery system of claim 24, further comprising:

a graphical user interface, coupled to said monitor, to enable a user to view efficiency data produced by said monitor and to use said efficiency data to control the synthesis process.

28. The delivery system of claim 1, further comprising:

a reaction chamber in fluid communication with said rotary valve.

29. The delivery system of claim 28, wherein a support surface is disposed in said reaction chamber.

30. The delivery system of claim 29, wherein said support surface is control pore glass.

31. A fluid or gas delivery system for performing a synthesis process, comprising:

a plurality of rotary valves; and

a manifold disposed between a fluid or gas supply and said plurality of rotary valves, wherein said manifold has an inlet and a plurality of outlets, and wherein said plurality of outlets are fluidly connected to an inlet port of each of said plurality of rotary valves.

32. The delivery system of claim 31, further comprising:

a plurality of distribution valves disposed between said fluid or gas supply and said manifold.

33. The delivery system of claim 31, wherein said plurality of rotary valves comprise between two to ninety-six rotary valves.

34. A valve for performing a synthesis process in a reaction chamber, comprising:

a passageway through which said fluid or gas flows; and

a check valve disposed in said passageway, wherein said passageway is in fluid communication with the reaction chamber.

35. A fluid or gas delivery system for performing a synthesis process, comprising:

a passageway through which said fluid or gas flows;

a swirl chamber disposed at one end of said passageway and configured to allow mixing therein; and

a reaction chamber disposed in fluid communication with said passageway.

36. The delivery system of claim 35, further comprising:

a check valve disposed in said passageway.

37. A system for concurrently performing a plurality of reactions, comprising:

a plurality of motors;

a plurality of rotary valves, each having a plurality of inlet ports and an outlet port, wherein each of said plurality of rotary valves has a valve rotor rotatingly disposed therein each of which is coupled to one of said plurality of motors;

a manifold disposed in fluid communication with at least one inlet port of each of said plurality of rotary valves to distribute fluids or gases to said inlet ports; and

a processor, wherein said processor controls movement and positioning of said valve rotor.

38. The system of claim 37, further comprising:

a plurality of distribution valves, wherein said plurality of distribution valves control the flow of fluids or gases to said plurality of inlet ports on said rotary valve.

39. The system of claim 38, wherein said processor further controls said plurality of distribution valves to allow or restrict the flow of said fluids or gases to said plurality of inlet ports.

40. The system of claim 37, further comprising:

a pressurized gas source to pressurize said fluids in said system.

41. The system of claim 37, further comprising:

a plurality of reaction chambers, each disposed in fluid communication with said outlet port of one of said plurality of rotary valves.

42. The system of claim 37, further comprising:

a plurality of output monitors, each disposed at an outlet of said plurality of reaction chambers for measuring the efficiency of the synthesis process in each reaction chamber, wherein said output monitors cause said system to terminate or modify said reactions.

43. The system of claim 37, wherein each of said valve rotors has a passageway formed therein, and a check valve disposed in said passageway.

44. A rotary valve, comprising:

a stationary housing with a plurality of inlet ports formed therein; and

a valve rotor rotatingly disposed in said stationary housing, wherein said valve rotor has at least one communicating port formed therein configured to align with said inlet ports of said stationary housing and has an outlet port formed on an opposing side of said valve rotor.

45. A rotary valve, comprising:

a stationary housing having a plurality of inlet ports formed therein; and

a valve rotor disposed within said stationary housing such that it is capable of rotating within said stationary housing, wherein said rotary valve has a passageway formed therein that is configured to allow mixing of fluids therein.

46. The rotary valve of claim 45, wherein the volume of said passageway is at least twice the volume of the smallest pulse of fluid delivered to said passageway.

47. The rotary valve of claim 45, wherein said rotary valve is in fluid communication with a reaction chamber, and wherein the ratio of the volume of said passageway to the volume of said reaction chamber is within a range of 2:1 to 1:1.

48. The rotary valve of claim 45, further comprising:

a plurality of O-rings disposed about said inlet ports.

49. The rotary valve of claim 45, further comprising:

a check valve disposed in said passageway of said valve rotor.

50. A rotary valve, comprising:

a stationary housing having a plurality of inlet ports formed therein;

a valve rotor disposed within said stationary housing such that said valve rotor is capable of rotating therein; and

a check valve disposed in said rotary valve.

51. The rotary valve of claim 50, wherein said check valve is disposed in said valve rotor.

52. The rotary valve of claim 50, wherein said check valve comprises a plurality of check valves, each check valve disposed in one of said plurality of inlet ports.

53. The rotary valve of claim 50, wherein said valve rotor has a passageway formed therein, and wherein said check valve is disposed in said passageway.

54. The rotary valve of claim 53, wherein said check valve is disposed in one end of said passageway adjacent said plurality of inlet ports.

55. A method for automated fluid delivery, comprising the steps of:

(a) sequentially adding two or more fluids to a mixing chamber such that said fluids are allowed to mix in said mixing chamber; and

(b) delivering said fluids to a reaction chamber by adding additional fluid or a gas to said mixing chamber, wherein said reaction chamber is fluidly connected to said mixing chamber.

56. The method of claim 55, wherein said automated fluid delivery is for performing a synthesis process.

57. The method of claim 55, wherein said step (a) comprises:

(i) introducing a short pulse of a first reagent into said mixing chamber;

(ii) introducing a short pulse of a second reagent into said mixing chamber; and

(iii) repeating steps (i) and (ii) until a predetermined amount of said first and second reagents have been introduced into said mixing chamber.

58. The method of claim 55, wherein said mixing chamber comprises a passageway.

59. The method of claim 55, wherein said mixing chamber comprises:
a passageway; and
a swirl chamber disposed at one end of said passageway.

60. The method of claim 55, wherein the volume of said mixing chamber is at least twice the volume of the smallest pulse of reagent delivered to said mixing chamber.

61. A method for performing a reaction, comprising the steps of:

(a) creating an event matrix for performing said reaction, said event matrix having a plurality of events;

(b) rotating a rotary valve to a first destination based on a first numerical value in said event matrix for a first event of said plurality of events;

(c) energizing a distribution valve based on a second numerical value in said event matrix for said first event, after said rotary valve has reached said first destination;

(d) executing a time dely for said distribution valve based on a third numerical value in said event matrix for said first event;

(e) de-energizing said distribution valve when said time delay is completed; and

(f) repeating steps (b) - (e) sufficient to perform said reaction provided by said event matrix.

62. The method of claim 61, wherein said event matrix is created based on a nucleotide sequence.

63. The method of claim 61, wherein said event matrix is created based on an amino acid sequence.

64. The method of claim 61, wherein said event matrix is based on a reaction for making an oligonucleotide.

65. The method of claim 61, wherein said event matrix is based on a reaction for making a peptide.

66. The method of claim 61, further comprising the step of:

(h) de-energizing said distribution valve prior to said step (b).

67. The method of claim 61, further comprising the step of:

(h) monitoring the efficiency of said reaction.

68. The method of claim 67, further comprising the step of:

(i) altering said event matrix based on the results of said monitoring step.

69. The method of claim 67, further comprising the step of:

(i) discontinuing said reaction based on the results of said monitoring step.

70. The method of claim 61, wherein said reaction comprises oligonucleotide synthesis.

71. The method of claim 61, wherein said reaction comprises peptide synthesis.

72. A computer program product comprising a computer usable medium having computer program logic recorded thereon for enabling a processor in a computer system to perform a reaction, said computer program logic comprising:

creation means for enabling the processor to create an event matrix for a desired reaction, said event matrix having a plurality of events;

rotating means for enabling the processor to rotate a rotary valve to a destination based on a numerical value in said event matrix for each event of said plurality of events;

energizing means for enabling the processor to turn on a distribution valve based on a numerical value in said event matrix for each event;

time delay means for enabling the processor to execute a time dely for said distribution valve based on a numerical value in said event matrix for each event; and

control means for enabling the processor to de-energize said distribution valve when said time delay is completed.

73. The computer program product of claim 72, wherein said control means further de-energizes said distribution valve prior to rotation of said rotary valve.

74. The computer program product of claim 72, further comprising:

calculating means for enabling the processor to calculate efficiency data for said reaction; and

display means for enabling the processor to display said efficiency data.

75. A method for monitoring synthesis of peptides or oligonucleotides, comprising the steps of:

(a) performing a synthesis process to create a desired sequence; and

(b) monitoring said synthesis process to determine an output sequence.

76. The method of claim 75, wherein said monitoring step determines an amino acid sequence for a synthesized peptide.

77. The method of claim 75, wherein said monitoring step determines an nucleic acid sequence for a synthesized oligonucleotide.

78. The method of claim 75, wherein a dye is added to one or more reagents used during the synthesis process.

79. A method for performing a reaction in a synthesis system, comprising the steps of:

(a) introducing a short, timed pulse of a first reagent in the system;

(b) introducing a short, timed pulse of a second reagent in the system; and

(c) repeating said steps (a) - (b) until a predetermined amount of said first and second reagents has been passed through the system.

80. The method of claim 79, wherein said first reagent and said second reagent are the same.

81. The method of claim 80, wherein said first reagent and said second reagent are detritylation reagents.

82. The method of claim 79, wherein said first reagent is a first part of a capping reagent, and said second reagent is a second part of said capping reagent.

83. The method of claim 79, wherein said first reagent is a phosphoramidite and said second reagent is an activator.

84. A method for arranging reagents about ports of a rotary valve for performing a synthesis process, comprising at least one of the following steps:

(a) positioning an activator at a port adjacent to each port containing a phosphoramidite;

(b) positioning a detritylation reagent adjacent a port containing a wash solution;

(c) positioning a first port of a two-port a capping reagent adjacent a second port of said two-port capping reagent; and

(d) positioning a wash solution adjacent one of said first and second ports of said capping reagent.

85. The method of claim 84, further comprising the step of:

(e) positioning a port for a gas adjacent to a home port position.

86. An arrangement of ports about a rotary valve for performing a synthesis process, comprising at least one of the following:

a port for an activator adjacent to each port containing a phosphoramidite;

a port for a detritylation reagent adjacent a port containing a wash solution;

a first port of a two-port capping reagent adjacent a second port of said two-port capping reagent; and

a port for a wash solution adjacent one of said first and second ports of said capping reagent.

87. The arrangement of claim 86, further comprising:

a port for a gas adjacent to a home port position.

88. A monitor for use in monitoring a reaction, comprising:

an optical sensor, said optical sensor having a first LED of a first wavelength, a second LED of a second wavelength both mounted on one side of tubing used in said reaction, and a detector assembly mounted on an opposite side of said tubing, wherein said first and second wavelengths are not the same;

a LED driver which controls illumination of said first and second LEDs;

a first narrow slit disposed between said tubing and said first and second LEDs;

a second narrow slit disposed between said tubing and said detector assembly and further disposed directly opposite said first narrow slit; and

a signal processor.

89. The monitor of claim 88, wherein said LED driver alternately turns on said LEDs.

90. The monitor of claim 88, wherein said first LED is a blue LED and said second LED is a red LED.

91. The monitor of claim 88, wherein said first and second narrow slits are parallel to and centered on an axis of said tubing, and wherein the width of said first and second narrow slits are chosen to match an inner diameter of said tubing, and wherein the length of said first and second narrow slits are equal to the combined diameters of said first and second LEDs.

92. The monitor of claim 88, wherein said detector assembly comprises:
a silicon diode optical detector; and
an internal amplifier.

93. The monitor of claim 88, wherein said LED Driver comprises:
an analog square wave oscillator which provides a low source impedance square wave signal to drive said first and second LEDs; and
a plurality of variable series resistors that allow outputs from said first and second LEDs to be matched to a standard value.

94. The monitor of claim 88, wherein said first and second LEDs are connected in parallel each with a series resistor with reversed polarity so that they are alternately turned on during a half cycle of the square wave.

95. The monitor of claim 88, wherein said signal processor comprises:
a synchronous demodulator for separating output signals from said first and second LEDs to generate a differential output;
a low-pass filter for filtering said differential output; and
an analog-to-digital converter card to convert said filtered, differential output into digital data for processing, wherein said digital

data is linearized using an empirical equation developed by correcting a voltage versus concentration curve.

96. A method for on-line monitoring of reactions, comprising the steps of:

(a) adding a predetermined amount of a fluid to a system;

(b) monitoring output data from the system; and

(c) providing said output data to a processor, wherein said processor analyzes said output data to determine if a reaction with said fluid is complete and optionally actively adjusts the amount of said fluid provided to the system based on said output data.

97. The method of claim 96, further comprising the steps of:

(d) calculating efficiency data from said output data; and

(e) terminating the reaction if said output data shows efficiency below a predetermined level.

98. A method for on-line monitoring of reactions in a reaction chamber during a synthesis process in a synthesis system, comprising the steps of:

(a) inputting a desired sequence into the system;

(b) adding a nucleotide or amino acid or derivatives thereof to said reaction chamber;

(c) monitoring output data from the system; and

(d) comparing said desired sequence to said output data to determine if the correct nucleotide or amino acid has been added.

99. A motor drive system, comprising:

a computer-controlled motor having a shaft extending therefrom; and

driver electronics mounted on said motor for converting a signal from a processor to a signal readable by said motor.

100. The motor drive system of claim 99, further comprising:

a zero position reference system mounted on said shaft of said motor.

101. The motor drive system of claim 99, wherein said computer-controlled motor is a servo motor with an encoder.

102. The motor drive system of claim 99, wherein said computer-controlled motor is a servo motor with a resolver.

103. The motor drive system of claim 99, wherein said computer-controlled motor is a stepper motor.

104. The motor drive system of claim 99, wherein said motor drive system is coupled to a system for performing a synthesis process.

105. A motor drive system, comprising:

a motor having a shaft extending from said motor, said shaft having a first end extending from an upper surface of said motor and a second end extending from a lower surface of said motor;

a zero position reference system mounted on said second end of said shaft; and

driver electronics mounted on said lower surface of motor for converting a signal from a processor to a signal readable by said stepper motor.

106. The motor drive system of claim 105, wherein said zero position reference system comprises:

a LED;

a silicon detector having a sensing element; and

a rotating aperture disk mounted on said second end of said shaft and having a radial aperture, wherein said LED is disposed on one side

of said radial aperture of said rotating aperture disk and said silicon detector is disposed on the other side of said radial aperture and directly opposite said LED.

107. The motor drive system of claim 106, wherein said zero position reference system further comprises:

an operational amplifier.

108. The motor drive system of claim 106, wherein a width of said radial aperture is equivalent to a width of said sensing element.

109. The motor drive system of claim 106, wherein said sensing element is mounted with a long axis radial to said shaft such that a maximum signal is available when said radial aperture of said aperture disk uncovers light from said LED.

110. A level sensor for a bottle containing a liquid, comprising:

a LED disposed about a curved portion of an outer surface of said bottle; and

a detector disposed opposite said LED about another curved portion of said outer surface of said bottle, wherein said detector detects a first amount of light emanating from said LED when fluid level in said bottle is above said detector and said LED and detects a second amount of light emanating from said LED when fluid level in said bottle is below said detector and said LED.

111. The level sensor of claim 110, wherein a signal from said detector assembly is sent to a processor to signal an alarm when the fluid level in said bottom is below said LED and said detector.

112. The delivery system of claim 1, wherein said delivery system is used to perform a reaction, wherein said reaction is selected from a group consisting of at least one of the following: oligonucleotide synthesis, peptide synthesis, polysaccharide synthesis, protein synthesis, protein purification, peptide purification, DNA purification, RNA purification, and biomolecule purification.

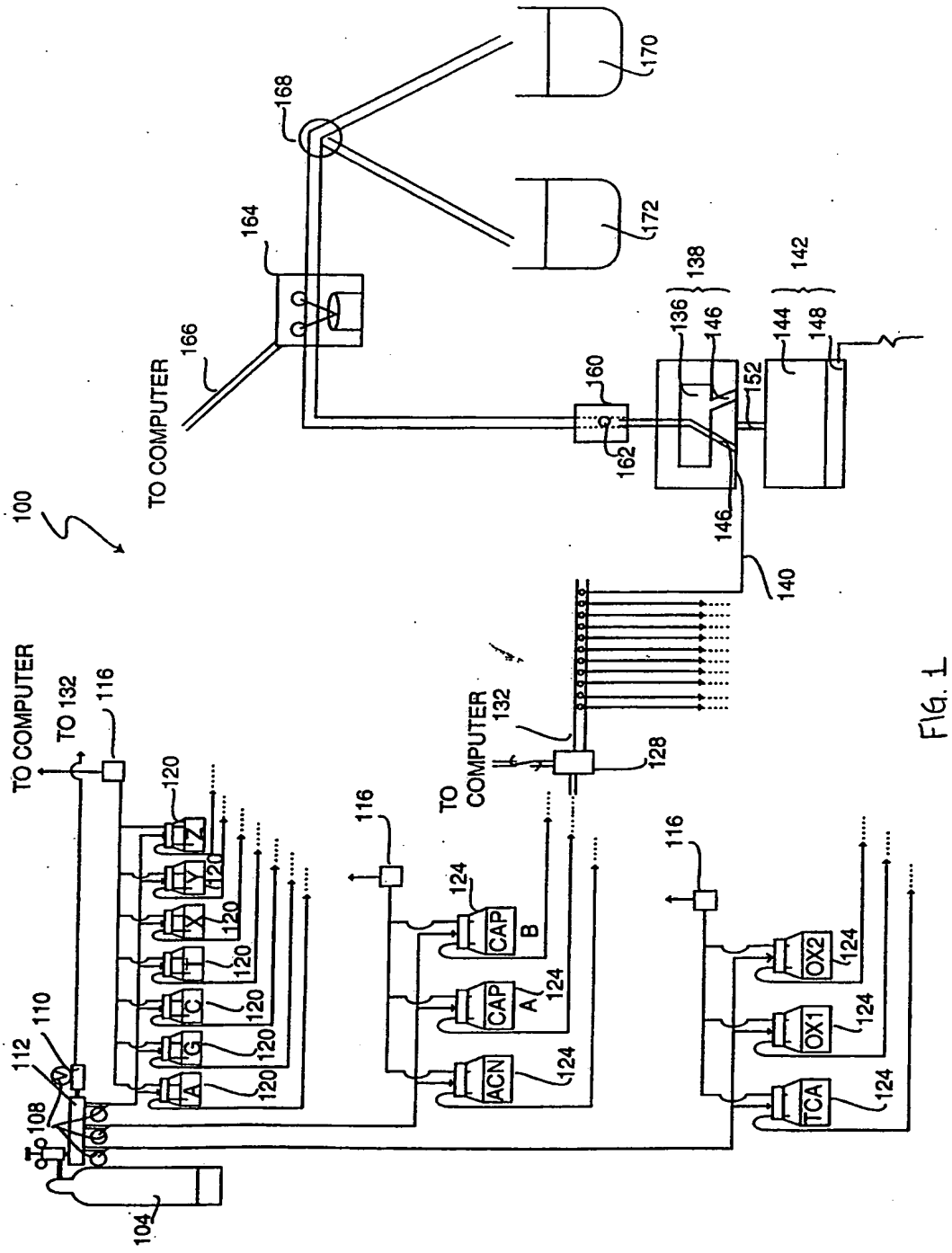
100
TO COMPUTER
166
164
168
170
172
138
142
136
146
144
148
160
162
152
146
140
TO COMPUTER
132
128
TO COMPUTER
TO 132
116
120
Z
Y
X
T
C
G
A
112
110
108
104
116
124
CAP B
CAP A
ACN
116
OX2
OX1
TCA
124

FIG. 1

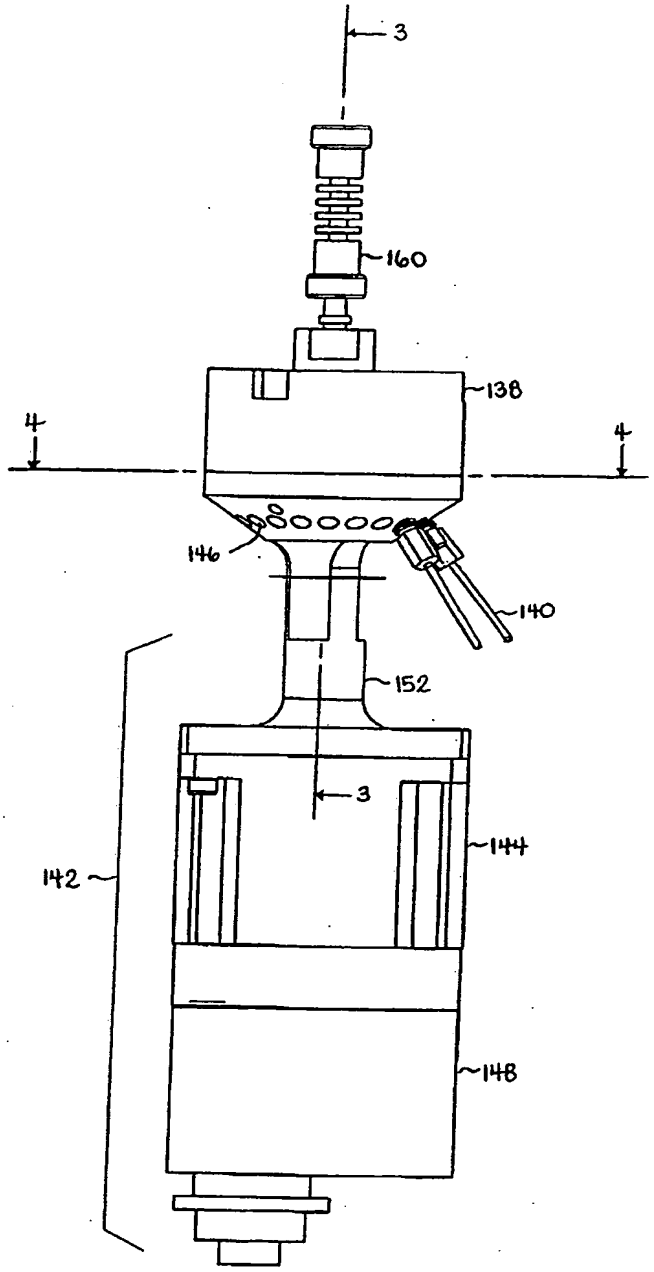

FIG. 2

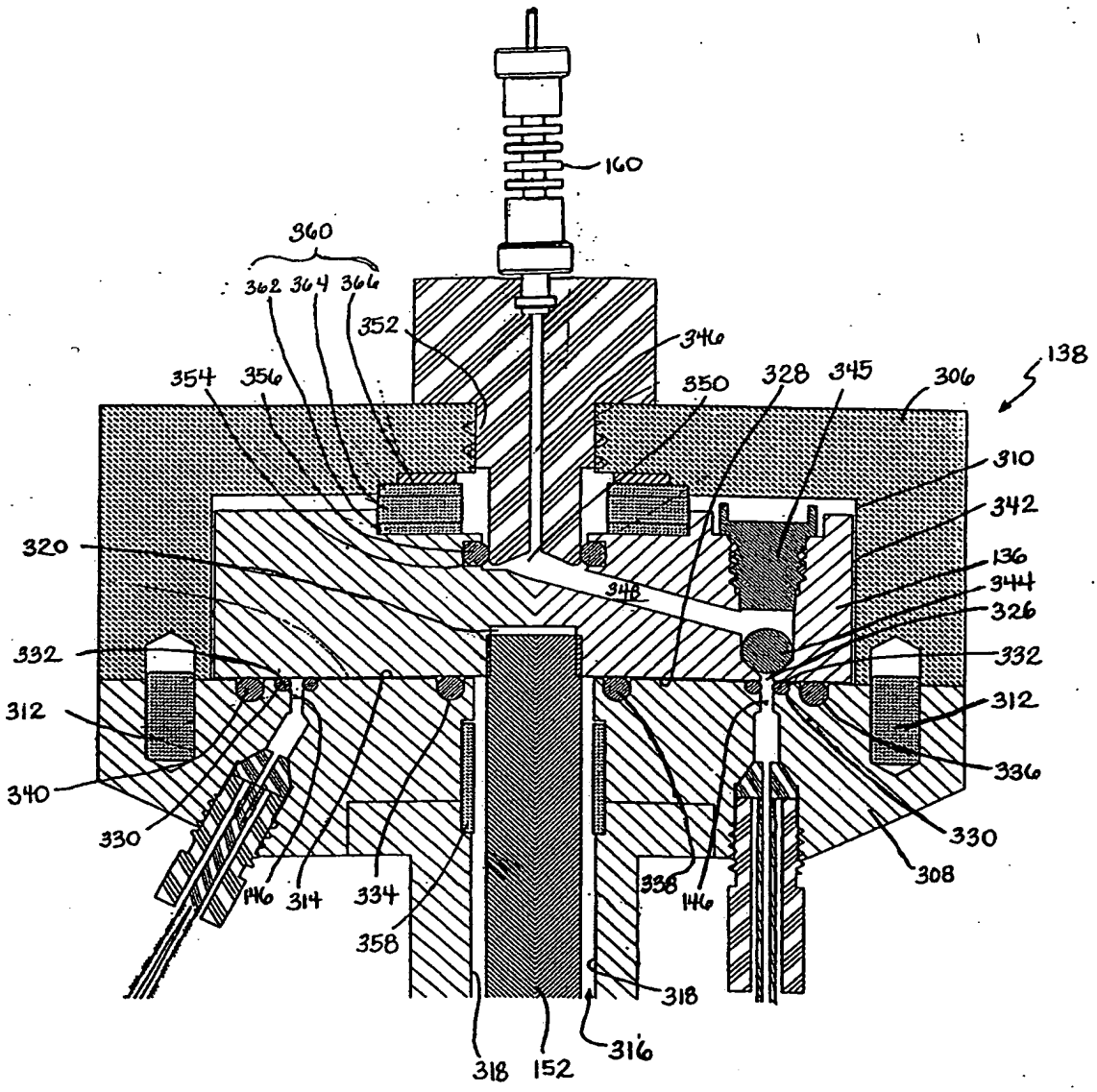
160
360
362
364
366
352
346
354
356
350
328
345
306
138
310
342
320
136
348
344
326
332
332
312
312
340
336
330
330
308
146
314
334
358
338
146
318
318
152
316

FIG. 3

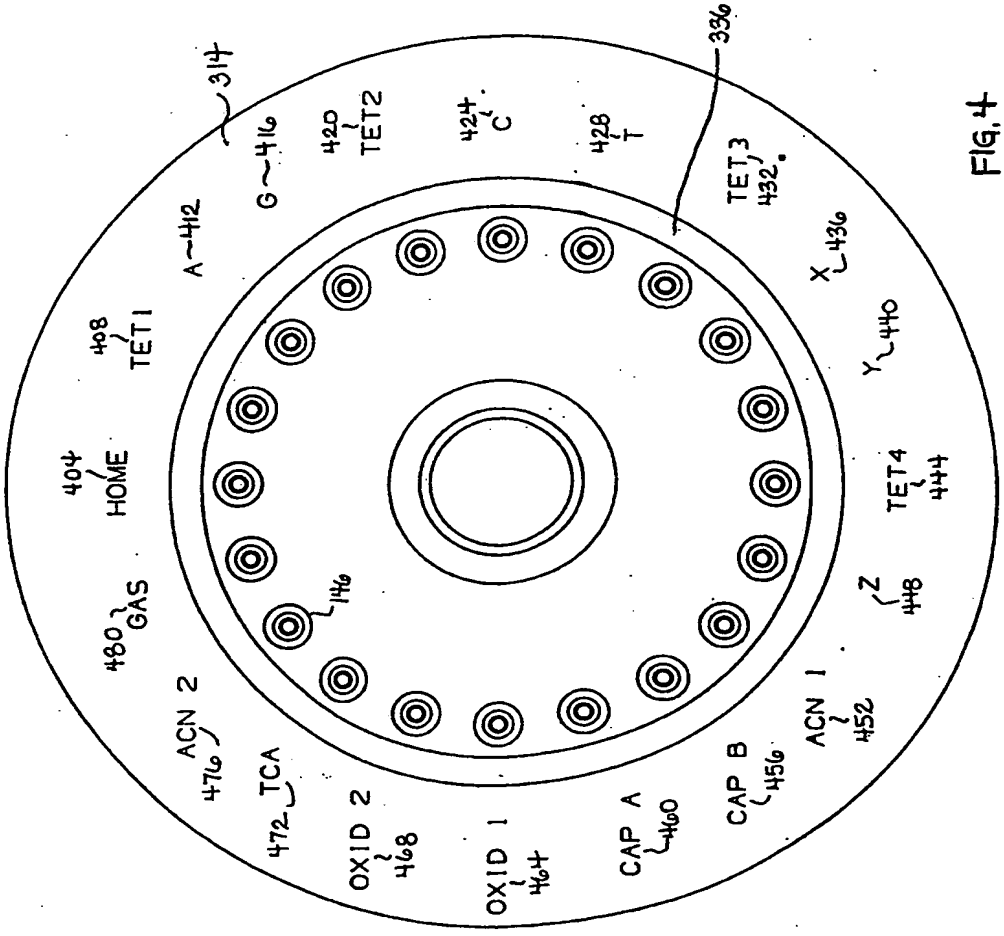
336
314
A~412
G~416
420 TET2
424 C
428 T
TET3 432
X 436
Y 440
TET4 444
Z 448
ACN 1 452
CAP B 456
CAP A 460
OXID 1 464
OXID 2 468
TCA 472
ACN 2 476
GAS 480
HOME 404
TET1 408
146

FIG. 4

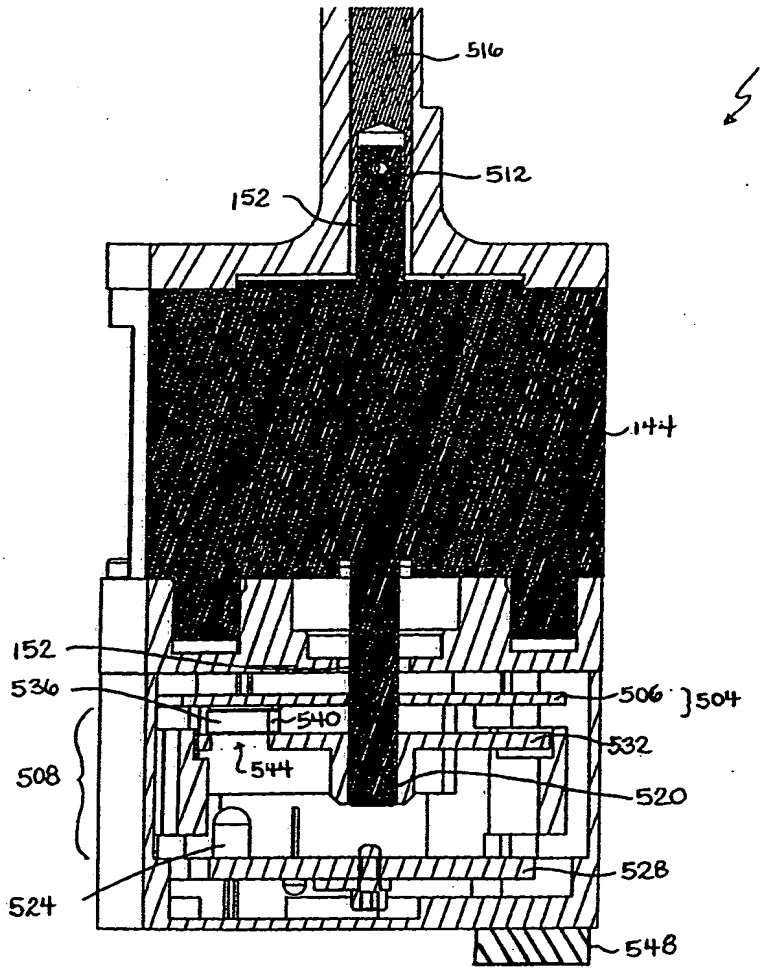
516
142
512
152
144
152
536
506
504
540
532
508
544
520
528
524
548

FIG. 5

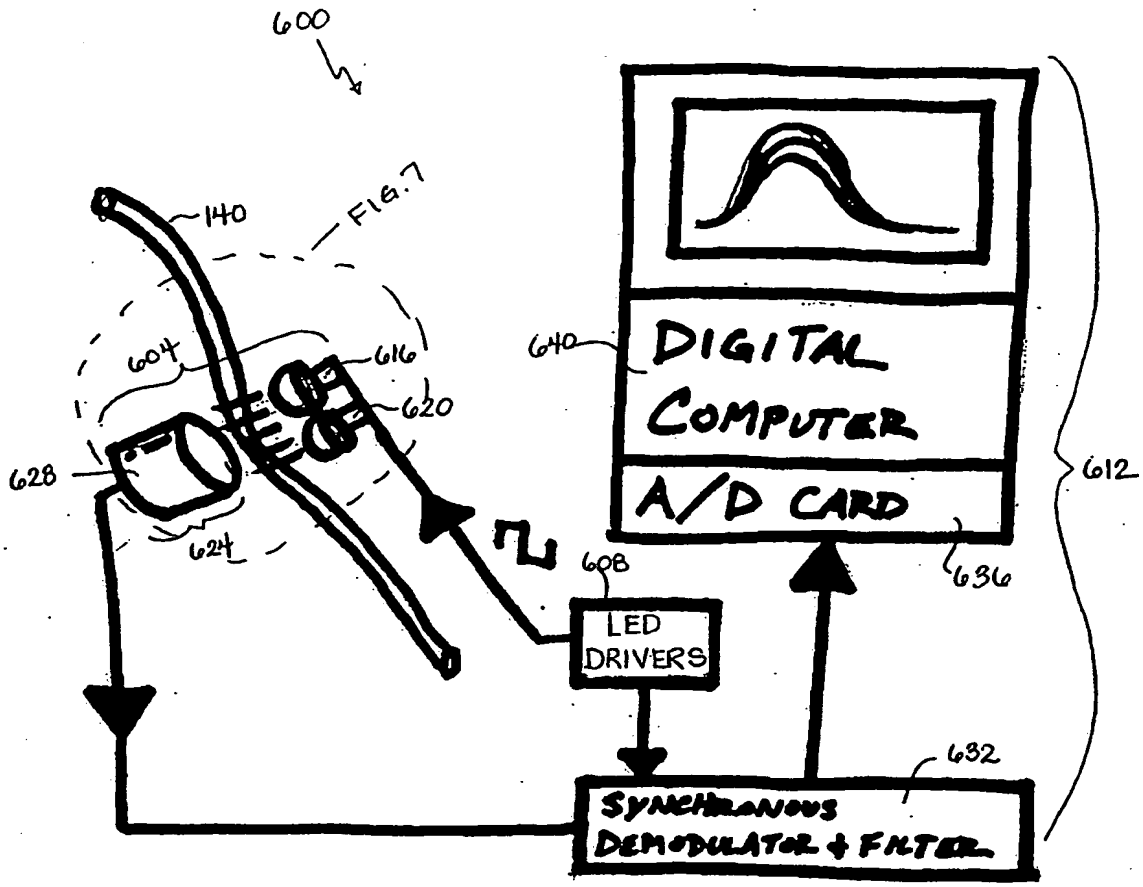
600
140
FIG.7
604
616
620
628
624
640
DIGITAL
COMPUTER
A/D CARD
612
636
608
LED
DRIVERS
632
SYNCHRONOUS
DEMODULATOR + FILTER

FIG. 6

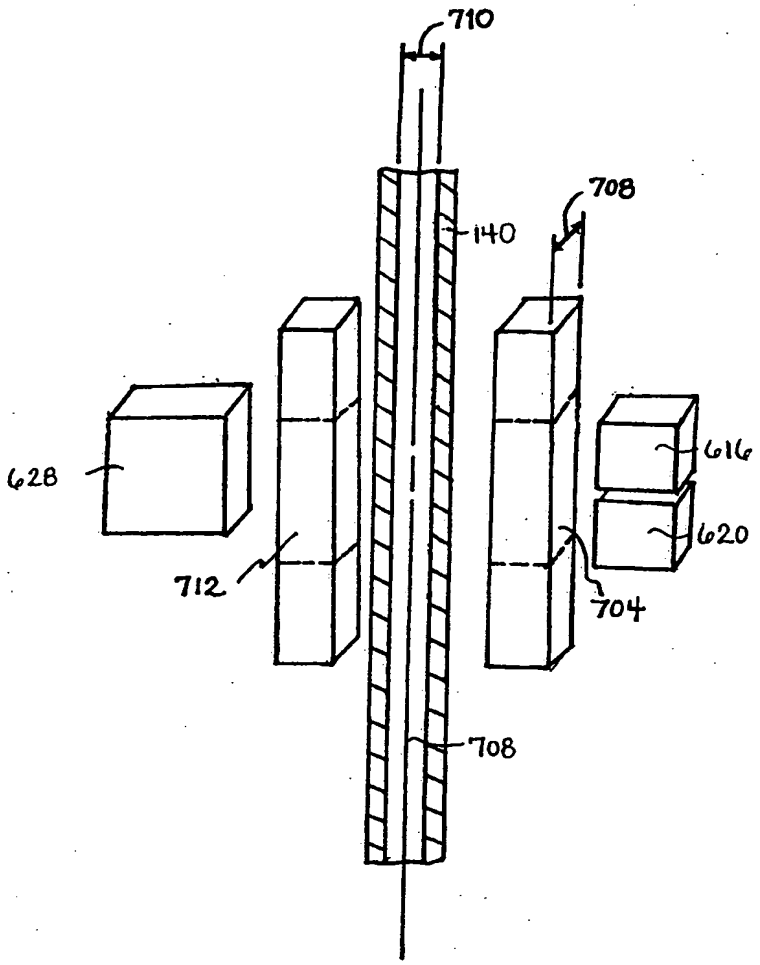

FIG.7

804 ~ 800

1	GACCTGTCAGDMT
2	CAGGTACTGDMT
3	ACGTTACTGTACNODMT
4	CGTATCGTACTDMT
5	GTTACTGGACTAGNODMT
6	CACTGGTACTDMT
7	ACGTACCGTNODMT
8	ACGTACGTCAGGDMT

FIG. 8A

812 ~ 808

1	GACTGTCCAG4SSSSSSSSSSSSSSSSSSSSSSSSSSS
2	GTCATGGAC4SSSSSSSSSSSSSSSSSSSSSSSSSSSS
3	CATGTCATTGCA5SSSSSSSSSSSSSSSSSSSSSSSSSS
4	TCATGCTATGC4SSSSSSSSSSSSSSSSSSSSSSSSSS
5	GATCAGGTCATTG5SSSSSSSSSSSSSSSSSSSSSSSSS
6	TCATGGTCAC4SSSSSSSSSSSSSSSSSSSSSSSSSSS
7	TGCCATGCA5SSSSSSSSSSSSSSSSSSSSSSSSSSSS
8	GGACTGCATGCA4SSSSSSSSSSSSSSSSSSSSSSSSSS

FIG. 8B

820 ~ 816

1	8, 4, 6, 10, 8, 10, 6, 6, 4, 8, 18, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2
2	8, 10, 6, 4, 10, 8, 8, 4, 6, 18, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2
3	6, 4, 10, 8, 10, 6, 4, 10, 10, 8, 6, 4, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2
4	10, 6, 4, 10, 8, 6, 10, 4, 10, 8, 6, 18, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2
5	8, 4, 10, 6, 4, 8, 8, 10, 6, 4, 10, 10, 8, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2
6	10, 6, 4, 10, 8, 8, 10, 6, 4, 6, 18, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2
7	10, 8, 6, 6, 4, 10, 8, 6, 4, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2
8	8, 8, 4, 6, 10, 8, 6, 4, 10, 8, 6, 4, 18, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2

FIG. 8C

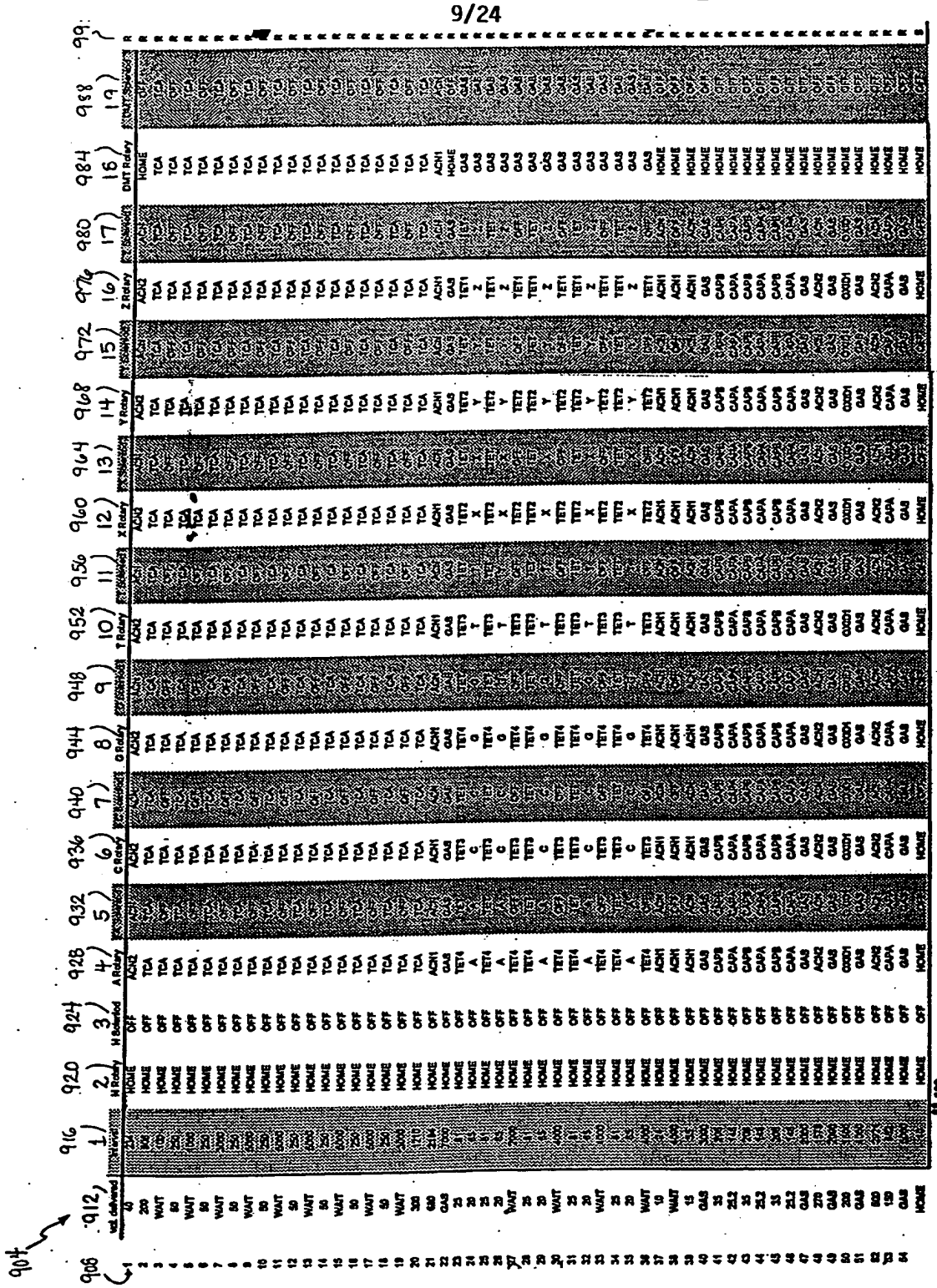

FIG.9

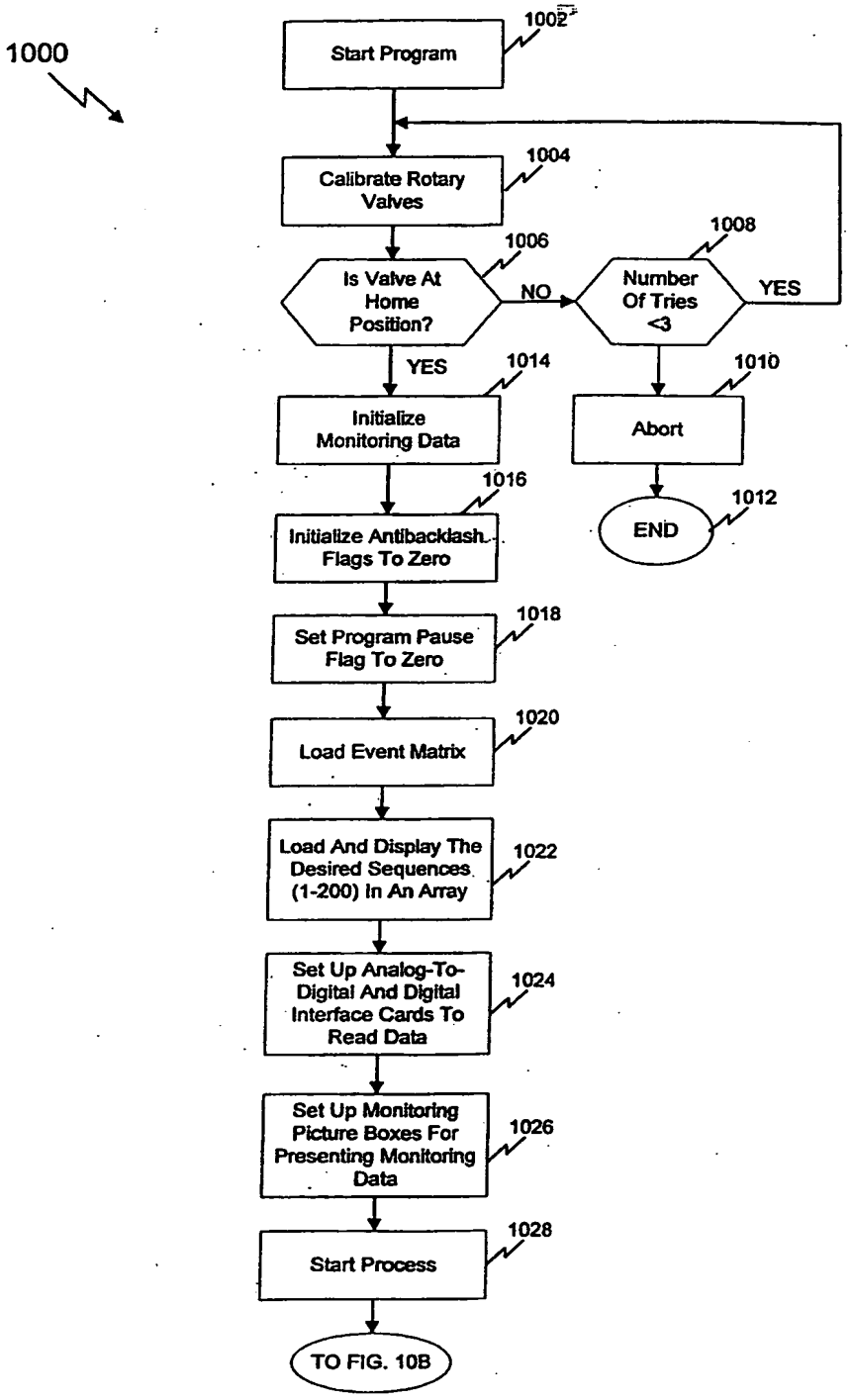
1000
1002
Start Program
1004
Calibrate Rotary Valves
1006
Is Valve At Home Position?
NO
1008
Number Of Tries <3
YES
YES
1014
Initialize Monitoring Data
1010
Abort
1016
Initialize Antibacklash Flags To Zero
1012
END
1018
Set Program Pause Flag To Zero
1020
Load Event Matrix
1022
Load And Display The Desired Sequences (1-200) In An Array
1024
Set Up Analog-To-Digital And Digital Interface Cards To Read Data
1026
Set Up Monitoring Picture Boxes For Presenting Monitoring Data
1028
Start Process
TO FIG. 10B

FIG. 10A

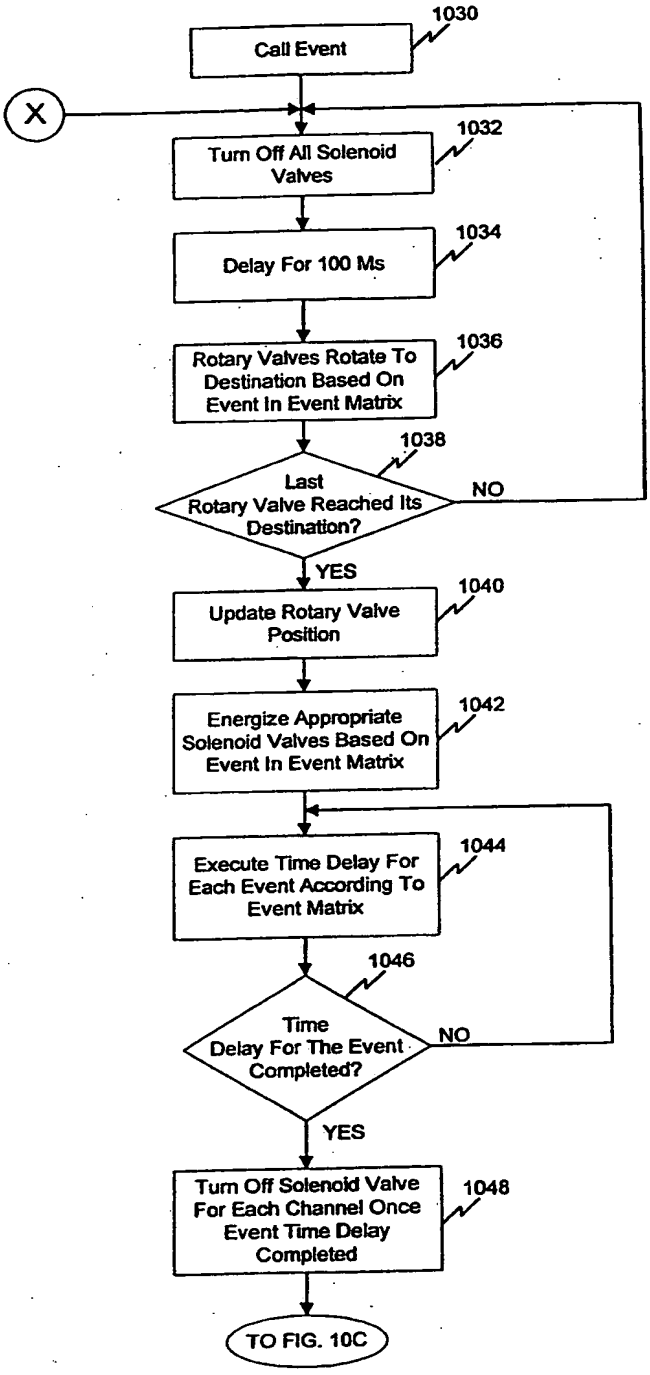
1030
Call Event
X
1032
Turn Off All Solenoid Valves
1034
Delay For 100 Ms
1036
Rotary Valves Rotate To Destination Based On Event In Event Matrix
1038
Last Rotary Valve Reached Its Destination?
NO
YES
1040
Update Rotary Valve Position
1042
Energize Appropriate Solenoid Valves Based On Event In Event Matrix
1044
Execute Time Delay For Each Event According To Event Matrix
1046
Time Delay For The Event Completed?
NO
YES
1048
Turn Off Solenoid Valve For Each Channel Once Event Time Delay Completed
TO FIG. 10C

FIG. 10B

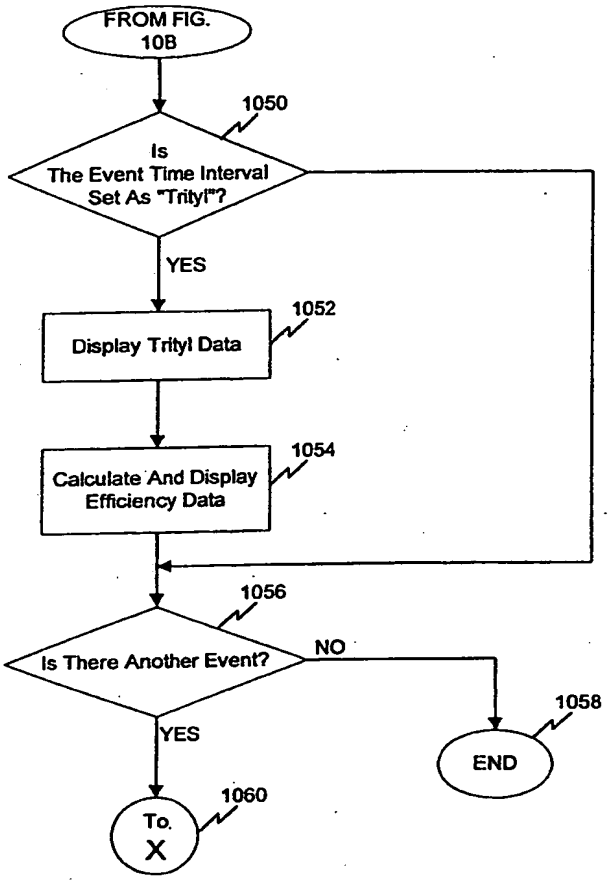
FROM FIG. 10B
1050
Is The Event Time Interval Set As "Trityl"?
YES
1052
Display Trityl Data
1054
Calculate And Display Efficiency Data
1056
Is There Another Event?
NO
1058
END
YES
1060
To X

FIG. 10C

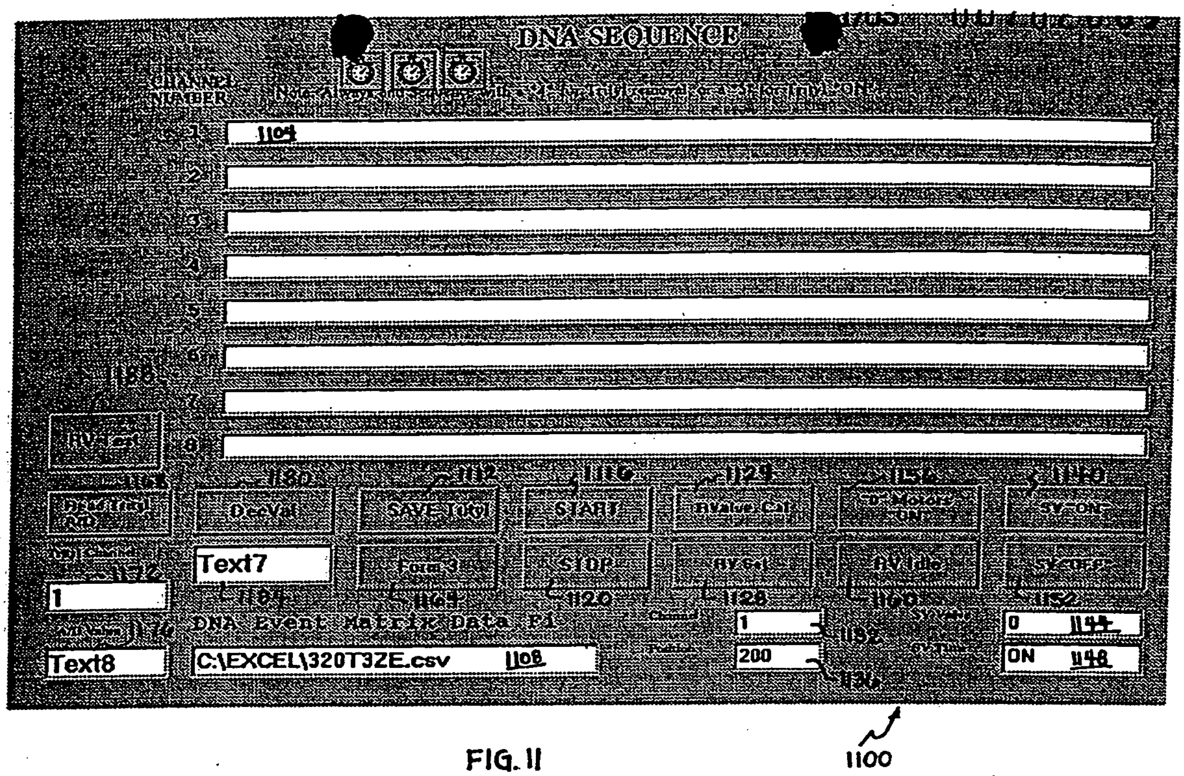
DNA SEQUENCE
1104
1188
1180
1112
1116
1124
1156
1140
DecVal
SAVE
START
RValue Cal
SV ON
Text7
Form 3
STOP
RV Set
RV Idle
SV OFF
1172
1184
1164
1120
1128
1160
1152
1
DNA Event Matrix Data Fi
1
1132
0
1144
Text8
C:\EXCEL\320T3ZE.csv
1108
200
1136
ON
1148
1100

FIG. 11

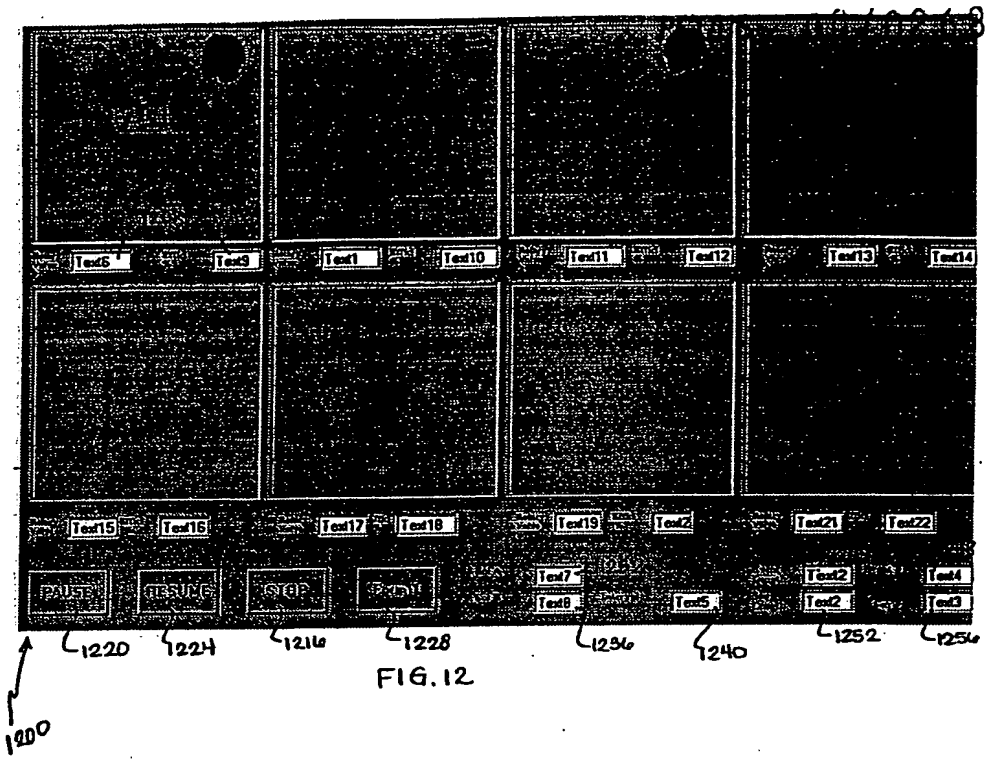
Test6
Test9
Test1
Test10
Test11
Test12
Test13
Test14
Test15
Test16
Test17
Test18
Test19
Test2
Test21
Test22
PAUSE
RESUME
STOP
EXIT
Test7
Test8
Test5
Test2
Test4
Test3
1220
1224
1216
1228
1236
1240
1252
1256
1200

FIG. 12

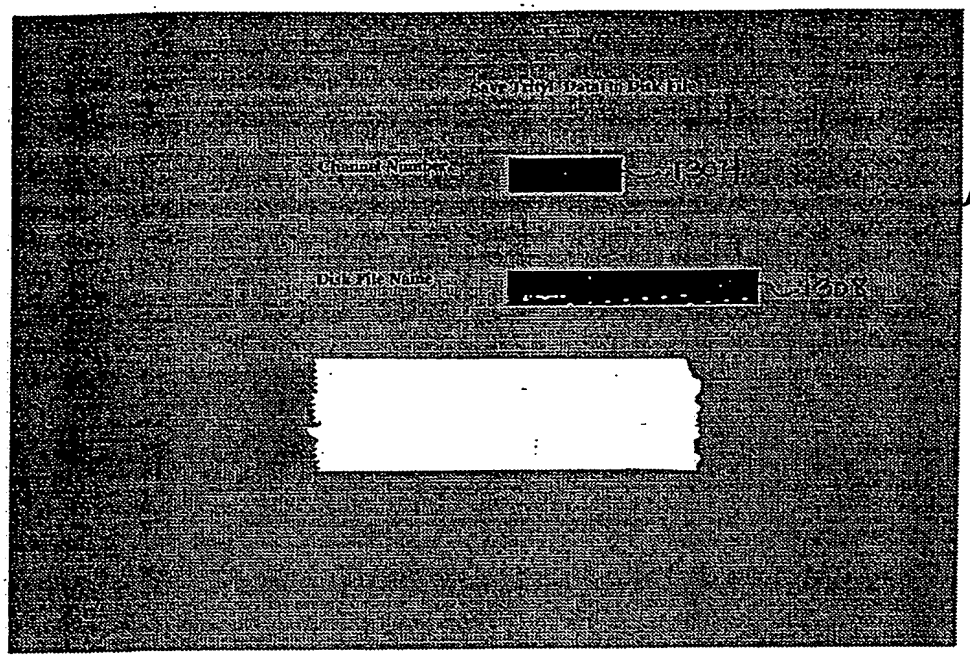

FIG. 13

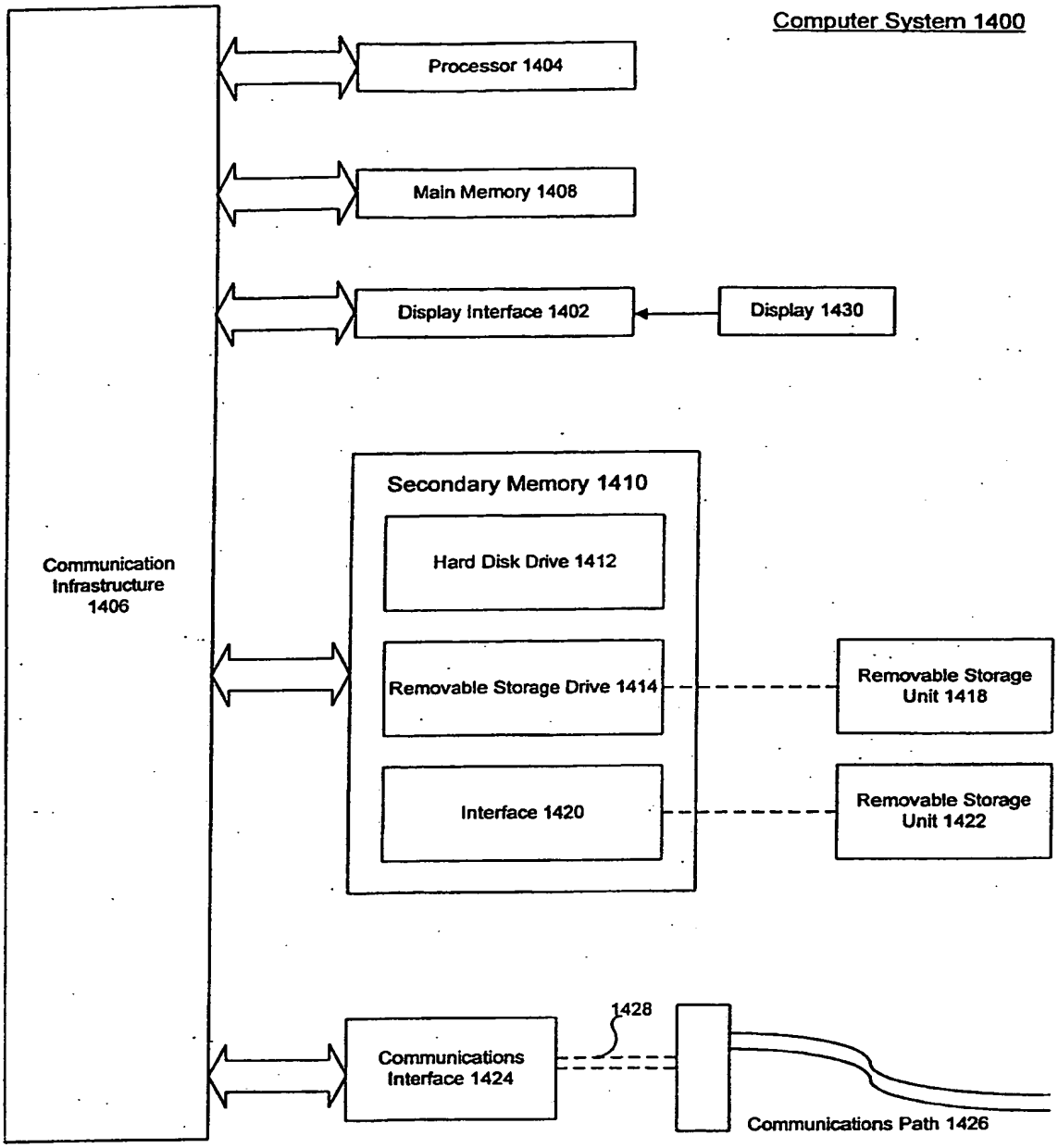
Computer System 1400
Processor 1404
Main Memory 1408
Display Interface 1402
Display 1430
Secondary Memory 1410
Hard Disk Drive 1412
Removable Storage Drive 1414
Removable Storage Unit 1418
Interface 1420
Removable Storage Unit 1422
Communication Infrastructure 1406
1428
Communications Interface 1424
Communications Path 1426

FIG. 14

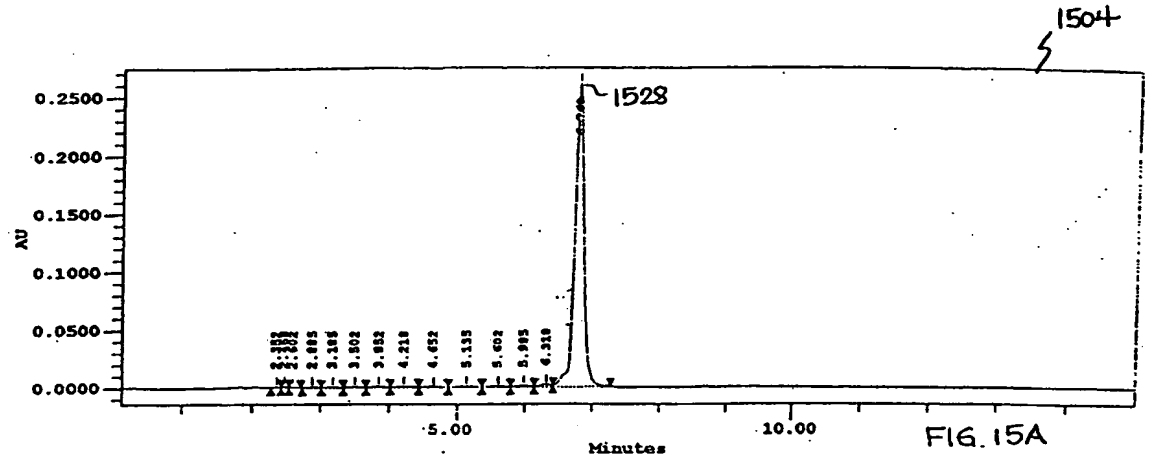

FIG. 15A

Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	% Area	% Height
1		2.352	3600	708	0.13	0.26
2		2.468	4984	1093	0.19	0.40
3		2.602	4301	700	0.16	0.26
4		2.885	5412	597	0.20	0.22
5		3.185	6949	707	0.26	0.26
6		3.502	7343	731	0.27	0.27
7		3.852	7842	731	0.29	0.27
8		4.218	9509	817	0.35	0.30
9		4.652	8954	717	0.33	0.26
10		5.135	11131	841	0.41	0.31
11		5.602	10624	897	0.39	0.33
12		5.985	12225	1137	0.45	0.42
13		6.318	20057	2033	0.74	0.75
14		6.768	2579753	260046	95.81	95.69

1512 1516 1520 1524 1508

FIG. 15B

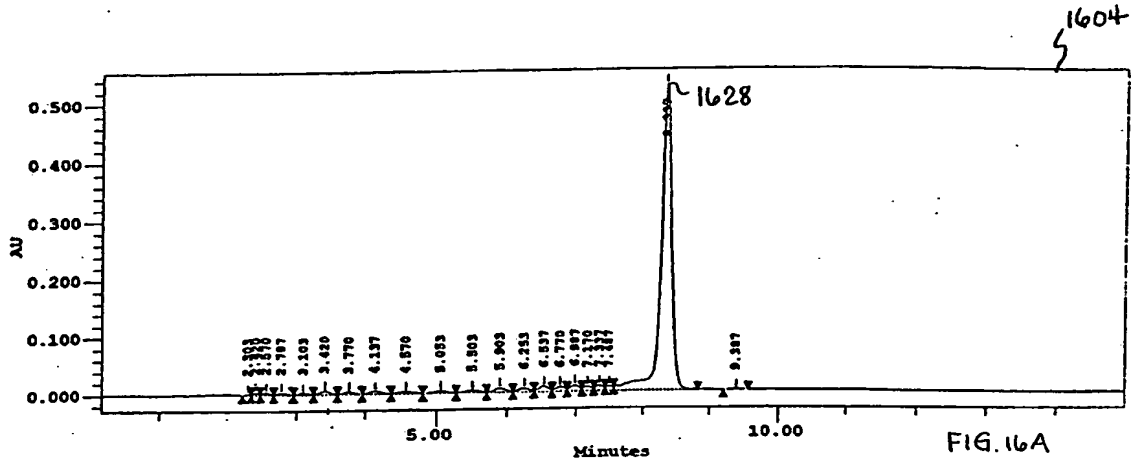

FIG. 16A

Peak Results

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	% Area	% Height
1		2.303	5251	1230	0.08	0.20
2		2.420	17291	3928	0.26	0.62
3		2.570	19310	3606	0.29	0.57
4		2.787	24104	2857	0.36	0.45
5		3.103	23691	2708	0.35	0.43
6		3.420	52135	5437	0.77	0.86
7		3.770	36651	3649	0.54	0.58
8		4.137	48115	4384	0.71	0.70
9		4.570	42864	3511	0.64	0.56
10		5.053	46817	3627	0.69	0.58
11		5.503	46187	3772	0.69	0.60
12		5.903	69985	6493	1.04	1.03
13		6.253	61003	5806	0.91	0.92
14		6.537	87803	9361	1.30	1.48
15		6.770	67435	7963	1.00	1.26
16		6.987	87707	10334	1.30	1.64
17		7.170	61557	7863	0.91	1.25
18		7.337	71329	9043	1.06	1.43
19		7.487	54889	7704	0.81	1.22
20		8.337	5805376	526231	86.16	83.46
21		9.387	8038	986	0.12	0.16

1612 1616 1620 1624 1608

FIG. 16B

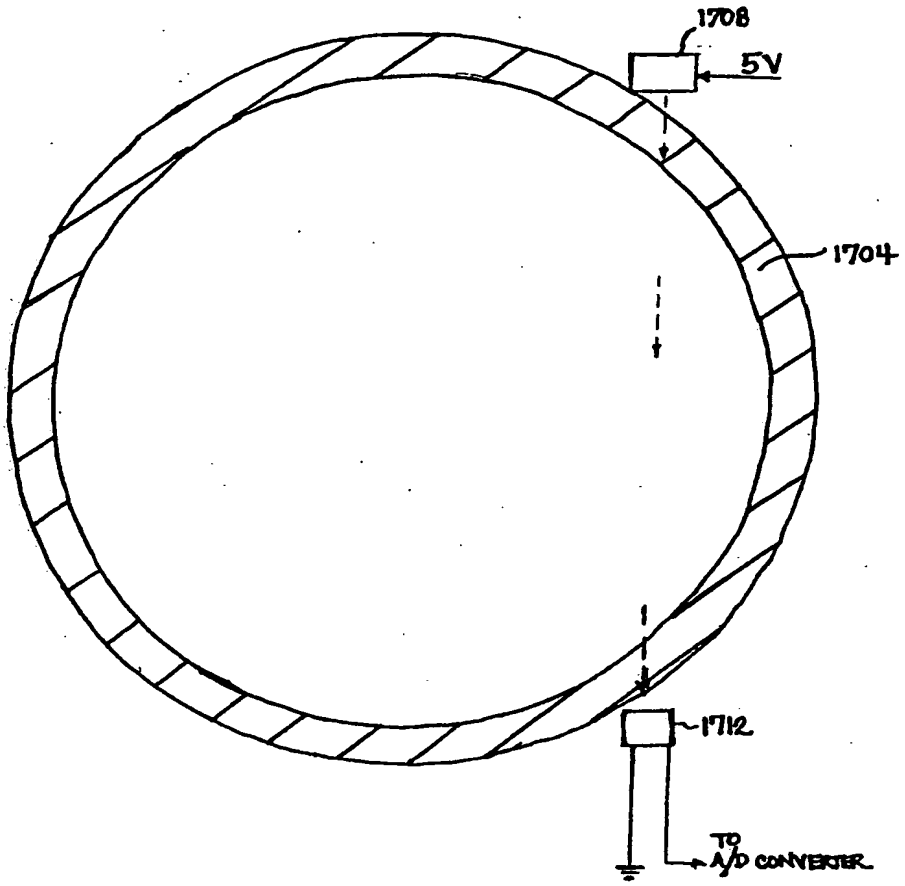

FIG.17

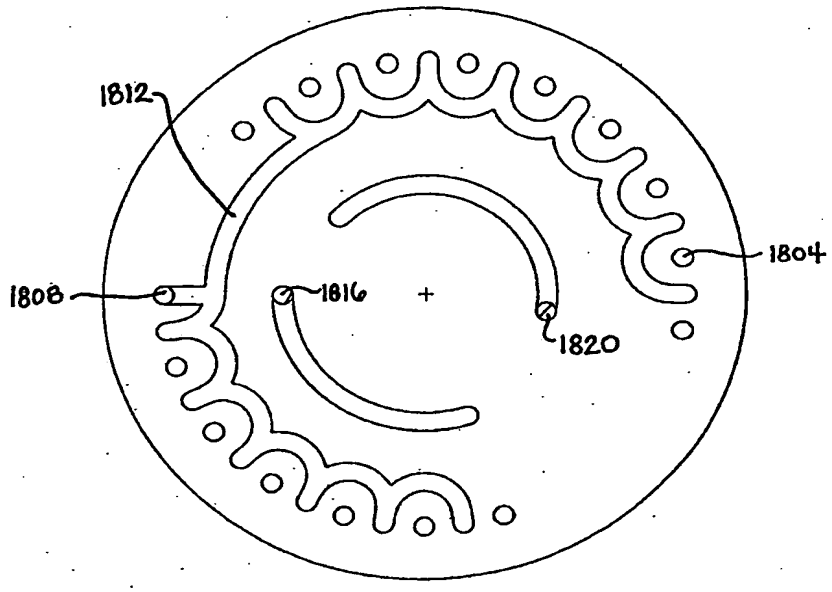

FIG. 18

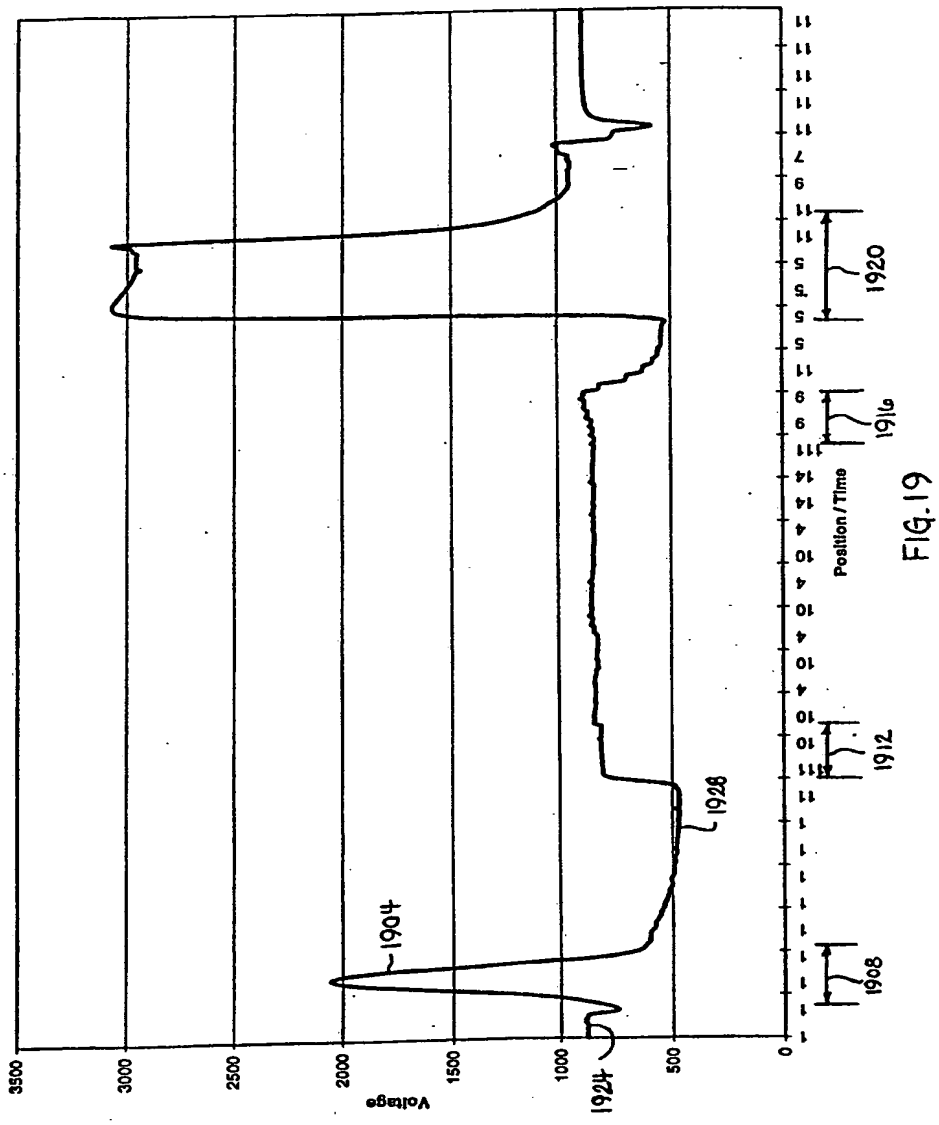
Voltage
3500
3000
2500
2000
1500
1000
500
0
Position / Time
1904
1908
1912
1916
1920
1924
1928

FIG. 19

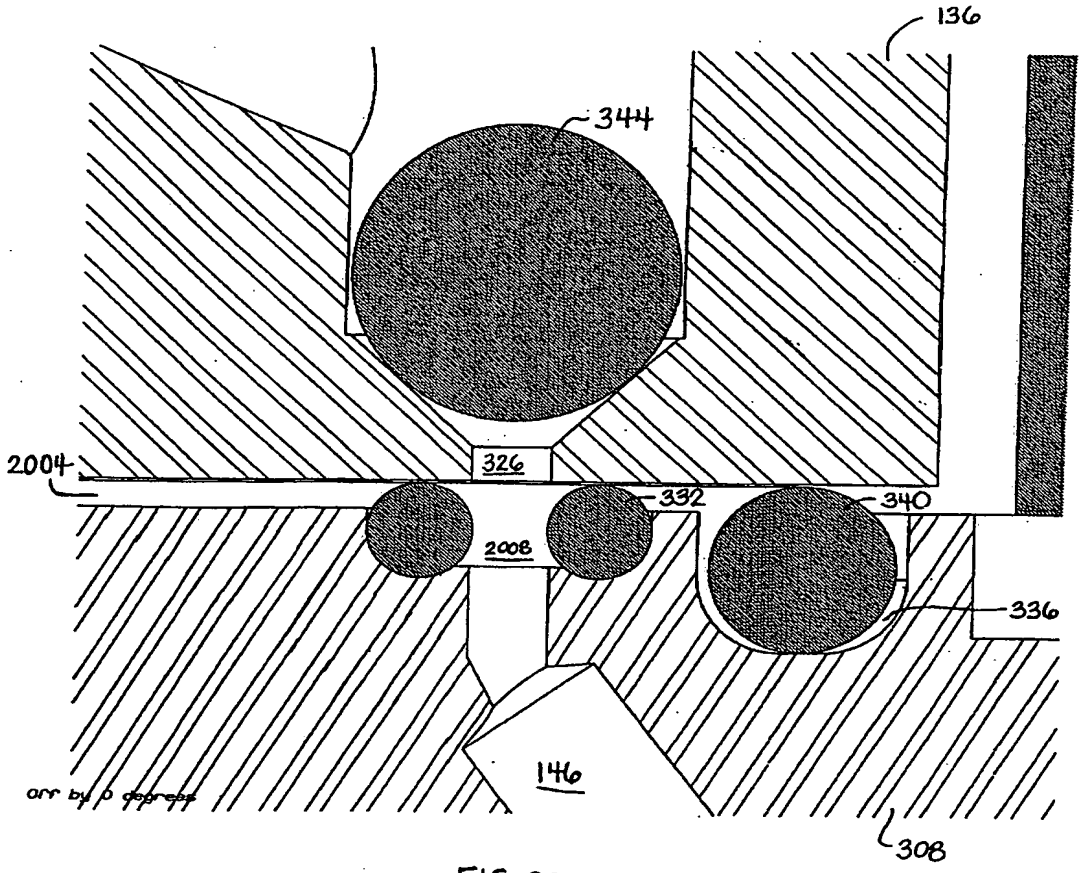

FIG. 20

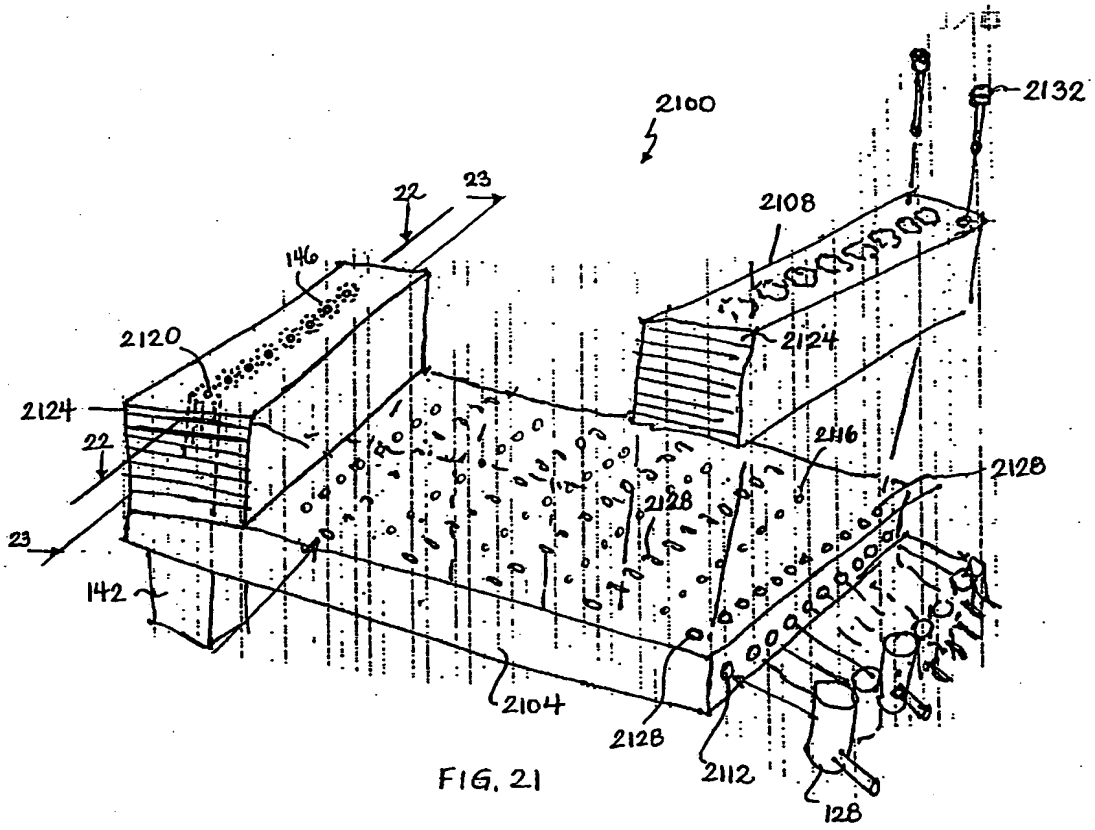
2100
2132
22
23
2108
146
2120
2124
2124
22
2116
2128
2128
23
142
2104
2128
2112
128

FIG. 21

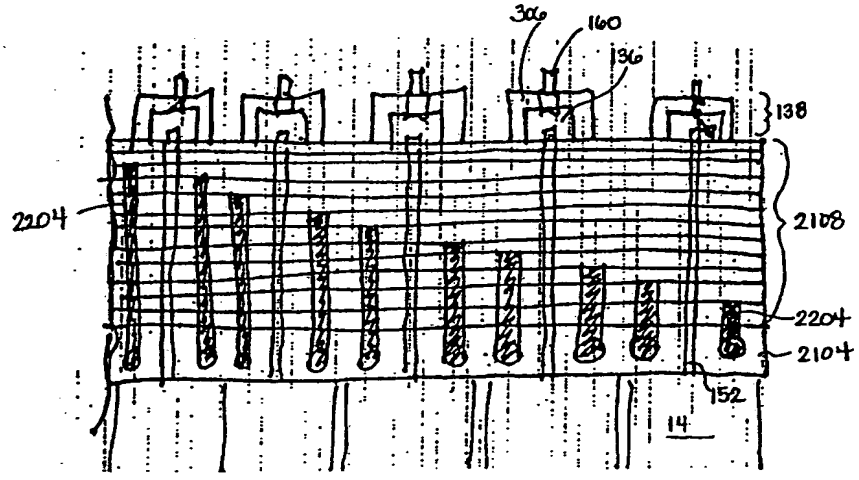
306
160
136
138
2204
2108
2204
2104
152
14

FIG. 22

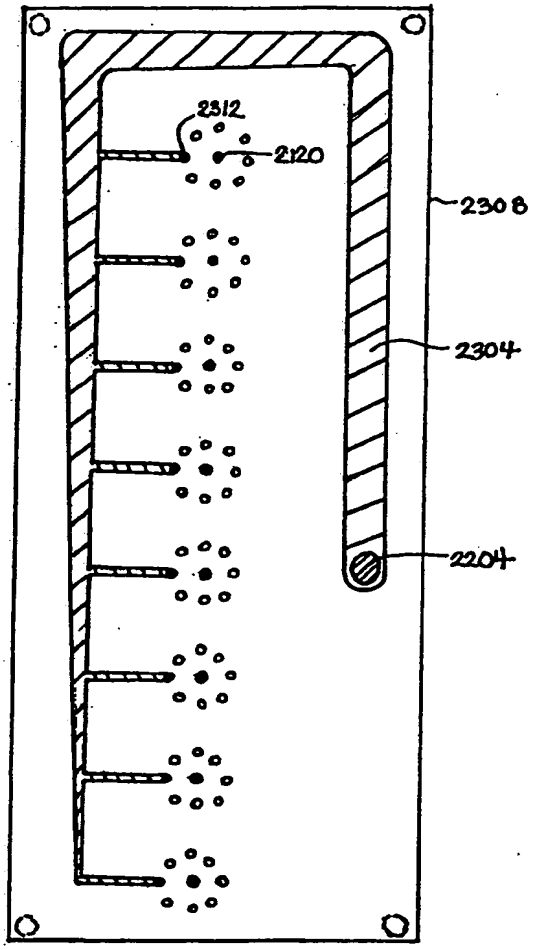

FIG. 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/02689

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :B01L 11/00; G01N 33/00; B32B 27/04
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/55, 62, 68.1, 82.01, 82.15, 103, 106, 131, 134; 435/ 287.2, 287.3; 436/55; 137/ 625, 625.11, 625.12, 625.15

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BRS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,368,823 A (MCGRAW et al) 29 November 1994, entire document.	55-87
Y	US 5,405,585 A (COASSIN) 11 April 1995, entire document.	1-60
Y	US 4,800,166 A (HORN et al) 24 January 1989, entire document.	1-60, 75-98
Y	US 3,687,163 A (NICKELS) 29 August 1972, entire document.	1-54
Y	US 4,458,066 A (CARUTHERS et al) 03 July 1984, entire document.	1-87, 112
Y,P	US 5,881,770 A (NEILL et al) 16 March 1999, entire document.	1-54, 84-87, 112

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
03 MAY 2000

Date of mailing of the international search report
16 MAY 2000

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Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/02689

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,811,218 A (HUNKAPILLER et al) 07 March 1989, entire document.	88-98
Y	US 4,863,849 A (MELAMEDE) 05 September 1989, entire document.	72-74
Y	US 4,538,640 A (ACKER) 03 September 1985, entire document.	1-54
Y	US 4,598,049 A (ZELINKA et al) 01 July 1986, entire document.	72-78
Y	US 5,002,384 A (TRACHTMAN) 26 March 1991, entire document.	88-95
Y	US 5,477,326 A (DOSMANN) 19 December 1995, entire document.	88-95
Y	US 4,671,941 A (NIINA et al) 09 June 1987, entire document.	1-60
Y	US 5,112,575 A (WHITEHOUSE et al) 12 May 1992, entire document.	1-87, 112
Y	US 4,354,180 A (HARDING) 12 October 1982, entire document.	110-111
Y	US 4,372,846 A (YAMAGAMI et al) 08 February 1983, entire document.	110-111
Y	US 4,690,165 A (LEYTES et al) 01 September 1987, entire document.	1-54

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/02689

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/02689

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/55, 62, 68.1, 82.01, 82.15, 103, 106, 131, 134; 435/ 287.2, 287.3; 436/55; 137/ 625, 625.11, 625.12, 625.15

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.
Group I, claim(s) 1-54, 84-87 and 112, drawn to rotary valve assembly.
Group II, claim(s) 55-60 and 79-83, drawn to a method for automated fluid delivery.
Group III, claim(s) 61-74, drawn to method and program for performing a reaction.
Group IV, claim(s) 75-78, drawn to a method for monitoring synthesis of peptides and oligonucleotides.
Group V, claim(s) 88-98, drawn to a monitor.
Group VI, claim(s)99-109, drawn to a motor drive system.
Group VII, claim(s) 110-111, drawn to a liquid level sensor.

The inventions listed as Groups I to VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of Group I is a check valve assembly which is not shared by any of the groups II-VII. The corresponding special technical feature of group II which is a method of adding two or more fluids to a mixing chamber and delivering the fluid to a reaction chamber and is also not shared by either groups I, III-VII. The special technical feature of group III is a method of creating an event matrix and is not shared by groups I-II and IV-VII. The special technical feature of group IV is a method of monitoring the synthesis process to determine the output sequence and is not shared by groups I-III and V-VII. The special technical feature of group V is an optical sensor and is not shared by groups I-IV and VI-VII. The special technical feature of group VI is a computer controlled motor and is not shared by groups I-V and VII. The special technical feature of group VII is a liquid level sensor and is not shared by groups I-VI, therefore holding of Lack of Unity of the invention is proper.